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TSUTSUGAMUSHI DISEASE (SCRUB TYPHUS) IN WORLD WAR II[1,2]

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Nowhere is medical and hygienic planning and execution so important to military operations as it is in the tropics. Striking figures that illustrate this are gleaned from the Burma campaign records of the British 14th Army where the ratio of disease to battle casualties regressed from 121 to 1 in 1943, through 19 to 1 in 1944, to a low 3.4 to 1 in the final stages in 1945 as preventive practice and medical facilities contingent upon a successful jungle campaign progressively improved. Conversely, statistics and fragmentary medical reports saved from this army's retreat from Burma in 1942 acclaim the dismal medical story of that unavoidable disaster.

World War II has brought much new information to light regarding the general subject of typhus fever in the tropics and has crystallized important phases of our prewar information. The term "tropical typhus" became a clinical catch-all for a time after World War I. Effectual separation of tsutsugamushi disease from this rickettsial disease complex, and its identification under various colloquial names, begun in the Thirties, was greatly accelerated by the pressure of military necessity.

The bibliography of tsutsugamushi disease, or scrub typhus as it was listed in reports of the various armed forces during the Asiatic-Pacific operations, 1941–1945, is already large and still growing. In some areas the disease was credited as a medical casualty producer second only to malaria and was more dreaded by the men.

Interference with actual combat operations was greatest on the Assam-Burma front where it was also learned through captured medical reports that the enemy likewise had his troubles with this disease, though strangely enough without recognizing until late in 1944 its identity with that of his homeland. The Americans and Chinese encountered several endemic and hyperendemic foci in North Burma. However, the Americans' total of 695 cases for the entire campaign in that area was exceeded by British casualties in each of 4 consecutive months, August to November inclusive, 1944, particularly along the Imphal jungle tracks and in the Kabaw and Chindwin Valleys to the east, indicating foci of major consequence in those areas. "Eighteen percent of a single battalion (2nd West Yorks) on the Tiddim Road got scrub typhus in two months, and in that time 5 percent of the total strength had died of it" (Audy). Episodes in the Australian forces were never as acute, though they encountered foci in North Queensland, Borneo, and New Guinea, notably in the Ramu Valley and the Aitape-Wewak area.

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[1] From the Rocky Mountain Laboratory, Hamilton, Montana, Division of Infectious Diseases, National Institute of Health.

[2] Presented 16 January 1948 before the New York Society of Tropical Medicine as the twelfth annual Theobald Smith Lecture.

Two episodes in the American Army in Dutch New Guinea and one in the British Army in Ceylon were especially notable because of the explosive results of relatively brief exposures. The epidemiology of these will be detailed later. Suffice it to say here that as a result of just 4 days of jungle exercise in Ceylon by a British Division, 756 patients were hospitalized, and the alarm created among the medical and general staff can well be visualized when "fever cases" by the hundreds reported to the dispensaries within a few days after return of the troops to their stations in India. American Task Force operations in the Schouten Islands resulted in a thousand cases in the first 2 months on Owi and Biak, reaching a total of 1,469 casualties in 6 month's time, while at Sansapor beach head the curve for weekly admissions on a thousand-per-year basis shot up to over 900 at the end of the second week, a rate higher for an individual episode than any yearly rate for all causes in the entire American Army in all theaters. In view of the strain on forward hospital facilities, and the severity of this disease, the noneffective curve for the Sansapor epidemic is indeed a prodigious one. The strain also on the few of us to whom the local units in both these outbreaks were looking for rapid help in control, will always remain a poignant memory of our Army activities. These two disasters alone provided a potential estimated loss of over 150,000 man days to the American Sixth Army.

Fortunately, enemy action at Sansapor was almost nil. Otherwise the story would have been very dismal indeed, for the First Infantry Regiment went ashore and bivouacked on abandoned native village areas and gardens which must have been veritable "ant hills" of infected mites. Subsequent patients with multiple eschars were plentiful, including one with the almost unbelievable number of 9, and these were carefully distinguished from scrub-itch or other lesions and infected abrasions so frequent in troops in the tropics. Southcott has reported an Australian patient with 4 simultaneous eschars in North Queensland.

Onset in the first patient at Sansapor, the Regimental Commander himself, was just 6 days after landing. In 20 days, 403 men of this regiment were hospitalized, including other key staff and noncommissioned officers—in addition to the commanding officer, there were the Executive Officer, the Chiefs of S1, S2, and S3, Headquarters Platoon Leader, Regimental Surgeon, Dentist, Liaison, Gas, and Orientation Officers, S4 of the 1st Battalion, 5 company commanders, and 13 lieutenants. Other scattering units of the task force were less heavily hit. This detail is given as an actual example of the disabling of a combat regiment as effectively as severe combat itself, for this could have been a strategic disaster for the beach head had the enemy been present in adjoining areas in force. Facilities of the 15th Portable and 29th Evacuation Hospitals were strained to the utmost to take care of this explosive outbreak. Attempts to evacuate patients in initial stages by flying boat were promptly discontinued, as unfavorable results quickly confirmed similar previous experience.

The eventual seriousness of this disease in Asiatic-Pacific operations was only vaguely anticipated in logistical planning, whereas the worry over its congener, epidemic typhus, in the European-Mediterranean forces was scarcely borne out. There were only 64 total cases of louse-borne typhus in the American Army during the entire war. This merits attention in passing, for classical typhus has been the scourge of military campaigns throughout recorded history and, as recently as 1915,

according to Strong, there were as high as 2,500 admissions a day to Serbian military hospitals, with perhaps three times that number among civilians. The demonstration of the efficacy of DDT powder in controlling lice, and hence an epidemic, during the typhus outbreak in early 1943 in Naples civilians will go down in the chronicles of military history as one of the outstanding hygienic accomplishments of this war. So effective were the louse powders in preventing lousiness in military personnel that it was difficult to obtain evaluation of the efficacy of prophylactic vaccination against epidemic typhus.

No such spectacular control methods were available against scrub typhus, as DDT powder would not prevent mites from biting. The most effective acaricides were developed too late in the war, and effectual indoctrination of the troops for their use was more difficult than it was for simple louse powders. No scrub typhus vaccine was developed, though that was emphasized as a goal early in the Pacific operations of all forces.

At the initiation of hostilities with Japan, there were many other lacunae in our knowledge of scrub typhus, though fundamental information had been contributed by investigators in Japan and Formosa, Malaya, the Netherlands Indies, and Australia.

Out of the recent war came some fine reports from the overburdened medical officers of the various forces who encountered this disease in outbreak proportions during their regular duties on the staffs of certain forward hospitals, or from other staff-assigned medical officers in various endemic areas. As a consequence the clinical and pathologic features have received special attention in print, adding some variation to the picture as it had been known since the disease was first described by the Japanese; only incidental mention of these features, therefore, will be made here.

The seriousness of the disease during the Asiatic-Pacific campaign stressed the need for specially trained personnel to assist in control operations and in the gathering of data "on the spot." Some of the most valuable additions to our knowledge of various phases of the problem have resulted from the special assignment of individuals or teams to different theaters in the endemic region.

The Australians placed several specially trained individuals in the field early in 1943, and the major contributions of McCulloch toward control will be mentioned. In October of that year a field team of the United States of America Typhus Commission was sent to New Guinea in response to a request from Southwest Pacific headquarters, and a year later another team was requested and sent to the Burma area. The U. S. Navy sent a medical research unit to the South Pacific Area which included an acarologist and mammalogist whose surveys contributed important data. In the meantime, British needs for similar help were voiced by Major General Thompson at a conference in February, 1944, immediately following the Ceylon epidemic, who stated "our job is definitely to keep men fit and in the fighting areas, and we must find out all the methods by which this disease is carried. We must form a team to correlate efforts in investigating research and to recommend prevention." Shortly thereafter, a team of British Army personnel and civilians was established in the India-Burma area.

Significant reports have been forthcoming from these various agencies. It is impossible to do justice to all in the time allotted here, and it is intended only to review some of the outstanding contributions made.

As in any disease in nature that is communicated to man by arthropods, there must be an interplay of 3 factors; namely, the vector species, a population of susceptible animal hosts, and the presence of the disease agent itself. Man is an accidental intruder in the natural cycle of tsutsugamushi disease, and so a discussion of these three factors relative to war-time investigations will precede presentation of incidence, distribution, epidemiologic, and control data. The terms tsutsugamushi disease and scrub typhus will be used interchangeably.

THE MITE VECTOR

Trombicula akamushi and *T. deliensis,* which are the only proved vectors to date, belong to the chigger-mite family, TROMBICULIDAE, and are so closely related by intergradation in the morphologic characters of individual mites in certain areas, that it is evident they have evolved from the same species complex.

Chigger mites are distinct from other mites parasitic on vertebrates in that only the first stage after hatching from the egg (i.e., the larva) is parasitic on a vertebrate host. The nymphal and adult stages are spent in a non-parasitic existence off the host. Until intensification of biologic studies late in the war by Wharton brought out the fact that the nymphs and adults are probably naturally predaceous on insect eggs and other minute arthropods, it was believed, following reports of the Japanese who succeeded in rearing only a very few to the adult stage, that they led a scavenger existence on vegetable and organic matter. Jenkins has now perfected techniques, using mosquito eggs as food for the nymphs and adults, whereby successive generations of North American chiggers can be reared in quantity. If this can be adapted to the rearing of the vector species, as seems likely, the way is opened for further important research. It should also provide the experimental answer to the important question of whether scrub-itch mites and other species can act as vectors.

Fuller (correspondence) has recognized 30 valid genera containing at least 310 known species of chigger mites. Roughly one-third of these have been described since 1940, the greater proportion from the Eastern Hemisphere, showing the stimulus provided by military surveys. The war focused attention on these minute pests, both because of their severe annoyance to troops in field exercises and in combat, and because of their potential and actual disease-carrying capacities. As a result many more specialists are studying them than before the war, and a number of institutions have assigned associated problems to graduate students.

The vectors have a decidedly reddish appearance in all stages, and when patches of larvae are seen attached in the ears of a host, it is a natural inclination to assume that they are full of blood. However, only lymph and tissue fluids from the subdermal layer are ingested, and the numerous references to blood-meals in the literature are incorrect. Since they normally attach to a host only once in a given generation as larvae, it is considered axiomatic that transovarial passage of the rickettsial agent between generations takes place. However, difficulties in techniques of rearing have prevented more than meager confirmation of this reasoning, both in early studies by the Japanese and more conclusively by the Burma team of the U.S.A. Typhus Commission. Infection in a small percentage of post-larval, nonparasitic stages has been reported in Japan by injection into monkeys of mites caught in nature. These and other circumstantial data are indicative of a low percentage of infection in mite populations in most endemic areas.

The question of an inactive phase of the pathogen in mites comparable to that observed in spotted fever-infected ticks has not been answered.

The American team in New Guinea confirmed natural infection in *T. akamushi* (under its synonymic name *T. fletcheri*) and furnished the first conclusive proof of infection in *T. deliensis* which was later abundantly confirmed by both the American and British teams in Burma. Mackie and his colleagues in Burma, moreover, labelled as "hyperendemic" areas of infection those areas in which ready recovery of strains from mite samples was accomplished, in addition to other criteria.

There has not been much light thrown on the important question of whether other mite species can act as vectors either to man, or as strict rat parasites providing possible inter-murid maintenance of natural infection. In addition to that of experimental rearing, two other difficulties have interfered with elucidation of this problem. Infection in samples of mites has been demonstrated by injecting pools of mites into Swiss mice, but the likely identity of the species used could only be determined by mounting an unused portion for microscopic identification. However, this does not insure that a few *akamushi* or *deliensis* may not have been present in the injected portion. Secondly, since *Trombicula* larvae remain attached to their animal hosts only for 3 or 4 days, they were never found in the subsequent eschar on a patient.

While emphasis as vectors is rightly placed on the "tsutsugamushi group" on the basis of present available information, we must be careful at this as yet early stage that we do not over-emphasize purely morphologic relationships among mites to the detriment of epidemiologic fact-gathering on other potential vectors. Circumstantial evidence reported by McCulloch and the U.S.A.T.C. team in the Pacific area cast suspicion on a few other species which require further investigation.

T. akamushi has been taken in Japan, Formosa, Luzon in the Philippines, in certain areas in North Burma, Malaya, and in parts of Dutch and British New Guinea. *T. deliensis* takes its name from Deli, Sumatra, and has been recorded in the Simla Hills and Assam, India, the Kunming district of South China, Ceylon, and the Maldive Islands, Java, Celebes, parts of Dutch and British New Guinea, certain of the Admiralty and Solomon Islands, and northern Queensland, Australia. The writer has specimens of supposed *akamushi* from Japan that are indistinguishable morphologically from *deliensis*. Surveys during the war widened our geographical horizons regarding these two species, and provided good reasons to suspect that *deliensis* should really be considered at most as a variety of *akamushi*.[3]

THE VERTEBRATE "RESERVOIRS" OF SCRUB TYPHUS

The word "reservoir" has been much abused in discussion of many diseases and has come more or less automatically to connote synonymy with any native susceptible animals. In spotted fever for example, in which the customary animal hosts of the tick vectors, such as ground squirrels, show only transitory infection in the circulation, it is the writer's belief the ticks themselves function as the real reservoirs.

In tsutsugamushi disease there is reason to suspect the reservoir role in nature is assumed chiefly by the mite vectors, and in a more transitory manner by susceptible

[3] In a report to the 1948 International Congress of Tropical Medicine with Mr. Glen M. Kohls, *deliensis* has been placed as such a variety.

native vertebrate hosts. Voles, *Microtus montebelli,* were early shown to carry natural infection in Japan, whereas in Formosa and other more tropical areas, various species and varieties of native rats have come into the picture. The U.S.A.T.C. team in New Guinea isolated 2 strains from *Rattus concolor browni,* and the Burma team from 8 *R. flavipectus yunnanensis* and, surprisingly, from 4 tree shrews, *Tupaia belangeri versurae.* This last animal is an insectivore and not a rodent, which broadens the zoological range of naturally infected mite hosts. The New Guinea team failed to confirm the previous claims of Heaslip of susceptibility (and natural infection) in the Australian bandicoot and of Gunther's belief that the New Guinea bandicoot is an important reservoir, though both of these marsupials are locally important hosts of vector mites, as is also, according to British surveys, the unrelated Indian bandicoot, a rodent. Kalra isolated a strain from the last animal (*Bandicota bengalensis*) as well as other strains from *Rattus rattus* var?, *Hydromys humei,* and a species of field mouse.

Experimentally-infected white rats were found by the New Guinea team to carry demonstrable infection in the blood for 22 days and in the brain at least 98 days, the longest period tried. Repeating this with laboratory-infected native rats, the British Burma team showed corresponding periods of 74 and 99 days respectively.

Many investigators since Hayashi, Hatori, and Walch, have drawn attention to the possibilities of birds either as carriers of infected mites to new areas or as potential "reservoirs" of the pathogen. Hatori reported 9 to 53 sparrows examined as naturally infected in Japan and experimental infection in several species of birds, but his criteria are rather confusing. In unreported experiments, the writer has demonstrated infection in circulation in English sparrows on the 10th day, pigeons on the 8th, chickens on the 4th, and turkeys on the 26th but not on the 41st day after injection. None of the serums taken at various periods in these birds devoloped OXK agglutinins or complement-fixing antibodies. In contrast is the recent record by Southcott in a nonendemic area of Australia of an OXK titer of 1/2560 in a wild, pied currawong in Queensland, the significance of which remains undetermined.

Since many prewar authors, and surveys during the war in many areas have shown various local birds to be infested with the vector species, the above information suggests that more attention should be paid to this phase of the problem. In some areas it is conceivable that birds could be an important supplement to rats in the "animal reservoir" complex and could also further complicate reliance on rat reduction for focal mite control.

Since mites do not take blood-meals, the relatively massive dosages of blood used to detect the pathogen in the circulation of susceptible mite hosts cannot be accepted without further difficult research as indicating that attached mites can become infected throughout the period of demonstrable infection in circulation. However, the suspected hosts are under heavy indictment and the degree of their guilt remains only to be proven with respect to the length of time they remain infectious for parasitizing vector mites.

THE ETIOLOGIC AGENT

This is the third of the basic factors in the disease complex. Various Japanese investigators, competitively, have suggested names for presumed pathogens responsible for this disease even before its distribution outside the Japanese Empire

was confirmed. A blood-protozoan affinity was first suspected and *Gregarina tsutsugamushi* was one of the suspected "agents" described as early as 1910. There has been dispute as to who was first in print regarding its true rickettsial nature. The first unquestioned binomial offered was *Rickettsia orientalis* Nagayo et al. 1930, but these authors and Hayashi had made reference to its apparent rickettsial relationship 7 years earlier. In 1920, Hayashi described a very pleomorphic, protozoan-like agent in the blood and tissues which he called *Theileria tsutsugamushi.* Breed (correspondence) has called attention to the writer to references in Japanese by Nagayo and other Japanese authors in which admission is made that Hayashi must have seen rickettsiae in some of his 1920 preparations. By acceptance of this evidence, his specific name by generic transfer becomes the one that will be most generally adopted, namely *Rickettsia tsutsugamushi* (Hayashi), in spite of his fantastic protozoan cycle and questioned techniques. This, therefore, antedates Ogata's *R. tsutsugamushi* (1931).[4] Many war-time reports followed the writer's previous acceptance of the name of Nagayo et al. as the first unambiguous, nomenclatorially acceptable reference to the etiologic agent. Sellard's earlier *Rickettsia nipponica* is considered inadmissible because of his reported cultivation of this agent.

Much of importance relative to the pathogen has come out of the war studies. Because of the attack upon preparation of a vaccine, and for improvement of specific serological diagnosis, stress was laid on recovery and comparative study of strains of *R. tsutsugamushi* from various areas. During studies in New Guinea and adjacent islands, the Philippines, and in Burma and India, strains were isolated chiefly from patients, to a less extent from mites, and a few from animal sources. Laboratory studies in animals were used in Malaya to show the identity of scrub typhus with tsutsugamushi disease of Japan as well as the relationship of pseudotyphus of Sumatra. Similar studies and serology were further employed to confirm identity of the disease in Queensland, New Guinea, the Philippines, Burma, and India. Enigmatic results which occurred, however, in the complement fixation were finally explained by Bengtson on the basis of antigenic differences between some strains which were wider than had been expected in view of the experience with other rickettsial pathogens. For example, Topping found that of 3 serums collected by the writer from members of a family infected on the same farm in Niigata, Japan, 2 from cases in 1944 were positive against a Burma strain antigen, and either positive in lower dilution or negative against a strain from New Guinea, while the third, a 1945 case, was positive against the latter antigen, negative against the former. Similar variation was found by Bengtson using serums from the Philippines and New Guinea. It was thus evident that 2 or more antigens with broad reactivities should be used in diagnostic fixation tests. A Japanese case, which we discovered in 1945 in a tributary valley of the Mogami River considerably below any known foci in central Yamagata, was also confirmed serologically (1 in 512 against New Guinea and zero against Burma antigens).

Smadel and his associates reported from embryonated chick eggs infected with a Burma strain a toxin capable of killing mice within a few hours. Antitoxins against

[4] The latest revision of Bergey's Manual of Determinative Bacteriology following the Bacteriological Code credits Ogata as the first generic revisor of Hayashi's species, but Ogata, as recently as December 1947, still claims independent naming of this agent, which hardly justifies this action. Kawamura was probably the first acceptable revisor.

this immune substance are so highly specific, however, that diagnostic neutralization tests against heterologous strains of *R. tsutsugamushi* are still not satisfactory.

These antigenic strain differences also have complicated vaccine studies and the adaptability of complement fixation for general laboratory use. The non-specific Weil-Felix test with *Proteus* OXK antigen will, therefore, continue to be the most practical and satisfactory for general use in connection with tsutsugamushi disease.

The war has unquestionably accelerated refinement of the laboratory tools which have been brought to bear on the various rickettsial agents. Though animals recovered from various strains of *R. tsutsugamushi* show complete immunity to heterologous strains, yet as Smadel has pointed out, the more delicate serological studies have shown differences of the order of those between epidemic and endemic typhus. These antigenic strain differences are not necessarily geographic, nor do they appear to be related to differences in the vector species. They do emphasize, however, that our present ideas regarding rickettsial species, far from being finalized, are still in a very formative stage and should be so regarded pending much more laboratory work with the whole rickettsial group of pathogens.

EPIDEMIOLOGY AND INCIDENCE IN THE ALLIED AND JAPANESE FORCES

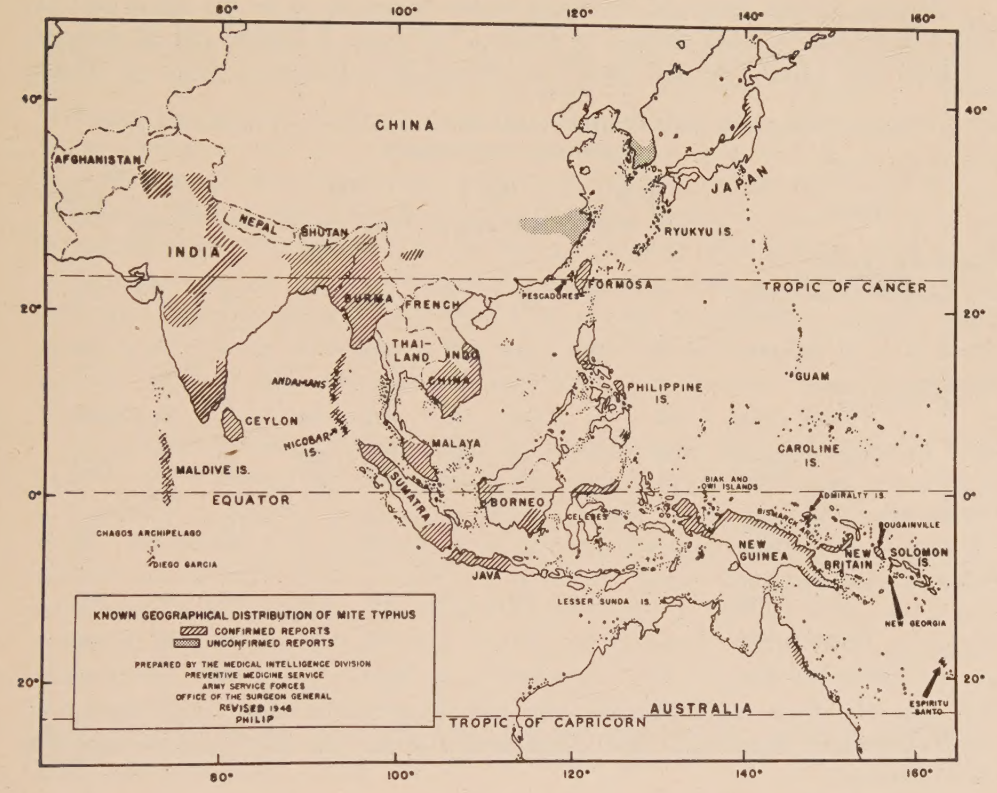
Field research was necessarily incidental to the chief concern of protecting the troops from tsutsugamushi disease. In the course of studies directed toward this end, much new epidemiologic information was accumulated in a relatively short period that normal commerical expansion in the tropics would have been years in revealing. And yet it can be said that most of these observations were an extension and confirmation of data previously recorded on a smaller and more diffuse scale. The same is particularly true of the clinical picture.

It has been aptly stated that troops are exceptional detectors of the presence of disease agents through mass exposure of non-immunes in new areas. Our information of the geographic distribution of tsutsugamushi disease in the Asiatic-Pacific area has been greatly enlarged both intensively and extensively as indicated on the accompanying map.

A few confirmed cases in American Army and Navy troops were contracted on Espiritu Santo of the New Hebrides which represents the most southeasterly location in the Pacific. The endemic area about Cairns and the Artherton Tableland in North Queensland, Australia, is a little farther south but still above the Tropic of Capricorn. In contrast, the disease is endemic well above the Tropic of Cancer in Japan and India, reaching nearly 40° N.L. in Honshu and above 30° in the Punjab and Kumaon Hills. Cases were reported in British-Indian troops in these locations of India, but the occupation of Japan after her capitulation was late in the season in the endemic areas, and no exposure of American forces in the restricted riverside foci was incurred.

In vertical distribution, Kalra of the Indian Army Medical Corps, reports *T. deliensis* up to 8,000 feet altitude in the Kumaon Hills and recovery of infection from them at 7,000 feet. Morishita had reported infection in Formosa up to 6,500 feet. Many cases were contracted by the Australians around 2,000 feet over the Kokoda Trail in New Guinea, and by the British in some of the Burma hills. Infection occurred in Americans in Northern Luzon at some 3,000 feet. The greatest exposure, however (and hence the majority of military cases), occurred at or just above sea level in the Pacific area.

Incidence.—Comparative figures for the various forces have been difficult to obtain. Available figures indicate a total of 6,861 cases in the U. S. Army, plus 613 cases in the U. S. Navy, approximately 6,730 in the British and Indian troops plus 79 ±reported in the British Navy, 3,188 in Australian troops, and at least 349 Chinese cases (Table 1). Rates on a thousand per annum basis are available only for some forces. However, rates actually mean but little when given on a theater rather than episode basis because of the mobile fluctuations in strengths and rapidity of local troop movements. For example, the U. S. Army statistics show admission rates on a 1,000 per year basis for the month of February 1943 for the Southwest



Pacific Theater of only 6.99, but the cases were almost all in bases north of Australia, giving an actual incidence of 25.73 in exposed troops. Consequently, rates have not been tabulated.

No racial differences were observed that were not correlated with exposure *per se*. In addition to white, Japanese, and Chinese cases, troops that were affected included East African negroes in India, Maoris from New Zealand, and New Guinea natives themselves (Noad).

Scrub typhus in the Japanese troops.—Only fragmentary data of a presumed considerable Japanese experience are available through captured documents. The writer has been unable to trace any central medical records in Tokyo giving overall incidence of the disease in the enemy fighting forces. Fragmentary evidence, however, through captured medical documents and interrogation of prisoners, has indicated that they encountered the disease in epidemic proportions in some parts

of both the Pacific and Burmese theaters. No certain record of actual diagnosis of tsutsugamushi disease was found, though on occasion similarity to flood-river fever in the homeland is mentioned where facilities for OXK agglutination tests were available. A captive Japanese medical officer interrogated by the writer through an interpreter reported a lack of these facilities in forward hospitals, though availability was indicated through the laboratory sections of their water purification units.

It was necessary in translations of their medical documents to distinguish "abdominal typhus," or typhoid, through the circumstances and clinical histories.

Specific records assignable to scrub typhus appeared to be the following: On the North Coast of New Guinea, at *Madang*: 65 cases, 2 deaths, and 32 doubtful, of what they called "Wewak fever"; at *Hansa Bay,* 3 cases, 1 death of "Hansa

TABLE 1.—*Cases and deaths of scrub typhus during World War II in military forces by theaters or commands*

Theater	1942		1943		1944		1945		Total Cases
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases	Deaths	
Southwest Pacific (plus South Pacific) Australia—Aus. (Army	2	1	160	8	152	2	31	0	345
(Navy			1	0	3	0			4
U. S. (Army	1	0	0	0	26	0	2	0	29
New Guinea and adj. islands)* Aus. (Army	186	40	1,870	112	602	78	181	27	2,839
U. S. (Army	33	0	941	48	4,357	165	80	19	5,411
(Navy			47	3	360	20	198	3	515
Philippines U. S. (Army					13	0	271	10	284
(Navy					36	1	62	1	98
South East Asia Command* U. S. (Army			56	0	610	28	301	30	967
Chinese (Army			17	1	202	26	130	13	349
Brit. Ind. (Army	+	+	537		3,801		1,062		5,400

* Including Assam but excluding the remainder of India, data for which are not available for comparative tabulation. All-India total cases from various sources for 1942–45, 420± cases; Ceylon, 1942–44, 799 cases; Maldives and Diego Garcia, 1942–44, at least 726 cases, plus approximately 79 cases in the British Navy; Andaman Islands and Nicobar, unstated number of cases (Navy or Army unknown).

fever"; *Yokapi,* 1 or 2 cases. Japanese soldiers were found sick by advance patrols in a native hut at the *Sansapor beach head,* but opportunity for questioning and further study was not afforded. Considering local endemicity, it was not unlikely some were ill with the disease. In Northern New Britain, near *Rabaul,* 40 to 50 cases were reported; in *Central Mindanao, P. I.,* OXK cases resembling flood-river fever reported; *Central Burma,* 140-odd cases in the vicinity of *Mandalay* and upper *Chindwin,* called "Burma eruptive fever," 7.4 per cent mortality in one area, some OXK positives, and reported recovery of a rickettsial strain in laboratory animals; *Imphal Plain,* cases near Tentha village were referred to as "Tentha disease"; numbers of Japanese infantry travelling through the *Somra Hills,* east of Kohima became infected, and "most of the patients contracting the fever died in about 14 days," suggesting a virulent focus of infection.

Attempts are being made to augment these fragmentary records by questioning of returned medical officers in Japan at the present time.

Mortality rates also give a more correct picture of variation in virulence in different areas by episode than when figured on a theater basis. Military outbreaks were, in general, surprising in their low death rates, none reaching the 55 percent recorded for Yamagata, Japan. Fatality in the Ceylon outbreak in 756 British-Indian troops was only 1.5 per cent, and in the 1,469 Owi-Biak epidemic in American troops was even lower—0.6 per cent; whereas in smaller outbreaks in American units on Goodenough Island and at Finchhaven, mortality reached 27.5 and 35.3 per cent respectively, in spite of prompt hospital care and with no added combat hazards.

Recovery was unquestionably prejudiced in cases with superimposed heavy combat fatigue, nutritional inadequacies through supply difficulties, complications of dysentery or malaria, and military necessity for too early evacuation. These factors were especially evident in members of Australian units infected during the Buna compaign, who had just been through the exhausting Kokoda Trail battles over the Owen Stanley Mountains, as well as in the early British fighting in Burma. Wingate's Special Force was flown into Northern Burma in March of 1944 and 50 patients had to be evacuated by air during the early stage of their illnesses; 15 of these patients died. It was thought that others died of the disease during combat without recognition in these early days. In contrast, the mortality dropped to 2.6 per cent in relieving forces later in the year, which was believed due more to better medical facilities and immediate hospitalization on location than to reduced virulence.

In general, levels of virulence tend to maintain about the same mortality from year to year in human victims in a given area regardless of fluctuations in incidence, a phenomenon roughly comparable also to North American tick-borne spotted fever. In spite of a marked decrease in cases of tsutsugamushi disease during the past decade in Japan, data provided the writer in Yamagata and Niigata indicated continuing high virulence. The drop from 39 deaths in 1938 to 17 in 1944 was a reflection of decrease in total cases and not in virulence. Data were, of course, not available over a long enough period in combat zones to establish reliable trends, but in the Cape Sudest-Oro Bay staging area of New Guinea over a 2-year period the over-all case fatality rate remained between 6 and 10 per cent in spite of fluctuating case incidence.

Comment on clinical features.—There was no apparent correlation between virulence and varying percentages of eschar, rash, and adenopathy. In New Guinea, 59 per cent of the Australians showed eschars compared with about 70 per cent in their cases in North Queensland. In the Ceylon outbreak, 85 per cent of the British-Indian cases showed primary lesions, whereas Tattersall reported only 11 per cent in outbreaks in Burma, which is still not as low as the 5 per cent in Malayans originally recorded by Lewthwaite and Savoor. Parker called attention to the complete absence of primary lesions in 195 British-Indian cases diagnosed clinically and serologically as scrub typhus in 1942-43 in the Calcutta area; these were contrasted to cases with eschar evacuated from Burma to the same hospitals. Rash also varied from 70 per cent to "almost all" in different areas and races, and not in consistent relation to the presence of eschar. Adenitis, especially in the region of the eschar, was the most consistent of the symptoms. Irons and Armstrong have provided a useful tabulated summary of clinical variation in reports of the various forces.

Enough atypical cases occurred during epidemics in different areas to make it probable that others, especially sporadic ones, remained undiagnosed in view of the lateness in appearance of OXK agglutinins. Wisdom born of experience in endemic areas dictated the handling of any febrile case, particularly if accompanied by adenitis, as suspect of scrub typhus, pending the ruling out of dengue and malaria by the duration of the fever and microscopic examination of the blood. Complete bed-rest as soon as possible after onset was found imperative, followed by symptomatic treatment thereafter in the absence of specific drugs. Expert and constant nursing care when available, especially for the severely ill, was repeatedly emphasized, though difficult to provide under combat conditions. Convalescent serum and penicillin, as tried both clinically and experimentally, were found to be of little benefit. Para-aminobenzoic acid came to the scene too late for military benefit, though a small series of cases treated by Tierney in Burma showed marked amelioration of symptoms and no mortality compared to an alternate untreated, more seriously ill series with 19 per cent death rate. Recent experimental studies by Smadel and his associates give hope that chloromycetin may prove of greater therapeutic benefit.

TABLE 2.—*Scrub typhus in American units on Owi island, Dutch New Guinea, reflecting graded incubation periods with successive dates of arrival, and periods required for counter measures to take effect*

	Date of arrival	Initial strength	Admissions during weeks ending (July and August): Jy 1	Jy 8	Jy 15	Jy 22	Jy 29	A 5	A 12	A 19	A 26
	June										
864th Avn. Engr. Bn. ..	13	766	5	17	19	4	6	3	5	4	
307th Airdrome Sq.	17	259	1	5	4	4	2	1	1		
308th Bomb Wing Hq. Det.	20	266	1	5	11	15	4	1			
929th Sig. Bn.	21	168	1	9	10	16	5	2	2		
	July										
63rd Bomb Sq.	6	210			2	5	26	36	12	4	
64th Bomb Sq.	13	?				3	28	37	16	2	3

Troop movements on short detail or in beach head landings in focal areas provided good opportunities to obtain data on minimal incubation periods. Logue records the shortest period of 4 days in the first of 230 cases in U. S. Naval personnel during the Cape Gloucester operation on New Britain, while one period of 6 days occurred at Sansapor. Most evidence indicated 7 to 21 days to encompass the usual periods, with an average of 11 to 12 days, for which periods there are several exact records. In table 2 this average period is well illustrated in graduated weekly admissions of cases in successive units locating in adjoining areas on hyperendemic Owi Island. Irons and Armstrong point out that the incubation period may be lengthened in mild cases. Difficulty in determining the exact day of onset when prodromal symptoms were vague and insidious was repeatedly encountered in various outbreaks.

Special features of the epidemiology during military operations.—One small epidemic investigated by the writer in New Guinea and reported clinically by Browning et al will be recounted in detail because it illustrates several features of the epidemiology peculiar to this disease. An American battalion had moved into, cleared, and occupied for 3 months without infection a knoll between two small

creeks on wooded slopes near Finchhaven. Six weeks after they moved out, 4 batteries of an Air Warning Battalion reoccupied this well-cleared area. Other staging units had continued to occupy adjoining areas on all sides and no cases of scrub typhus were previously reported in the vicinity, though 22 non-fatal cases had occurred sporadically in scattering units in other parts of the Base in preceding months. A sharp outbreak in 3 batteries of this new battalion resulted in hospitalization of 16 cases within a 3-week period in September 1944, after all had been on location for over 2 months. The battery with the most cases had been installed 88 to 112 days precedent to onset, though cases in epidemics usually began to appear within approximately 12 days of entering a new location. Determination of the cause of the delayed appearance in this battalion presented a special job of hygienic sleuthing, since the area had been well cleared and previously occupied, and the present occupants had done nothing in the way of jungle exercises in which adjoining unaffected units had not also participated. Careful questioning of patients and of members of the one unaffected battery finally indicated that these infections must have been contracted during a new job of clearing and early attendance of a small, outdoor theater on a slope some 100 yards below the previously cleared, infection-free company areas. Two cases occurred simultaneously in an adjoining unit across the creek, whose members had also attended shows at this theater. Ironically, the theater was dedicated to the first victim before it was known that he had probably contracted his infection as one of the first to work on the project.

Six deaths occurred in the 16 cases and most of the others were on the critically ill list, though hospitalization was immediate and adequate nursing and hospital facilities were available. The 37.5 per cent fatality in this small outbreak was the highest experienced in any American epidemic, though the "rate" for the Finchhaven Base by addition of the other 22 non-fatal cases at other times and places would mask the virulence in this one "island of infection." In contrast was a probable 17th case in the most affected battery. This patient had been hospitalized early in the outbreak with 2 days of "fever of undetermined origin" and discharged to duty without further diagnosis or development of any other symptoms. A serum sample of this man after 21 days of full duty had a high OXK titer.

Rats trapped in the area carried few mites, but these included *T. deliensis*. No free mites could be found on careful search in the theater area within a month after it had been cleared.

There have been other references to very mild febrile infections without other symptoms. Williams et al refer to ambulatory Australian cases diagnosed only after Weil-Felix tests were done, while Irons and Armstrong mention mild cases that remained on duty for days after the first vague symptoms; some were not hospitalized until an esthenic state had developed which was not usually seen until early in convalescence. This was corroborated by recovery of the first non-fatal strains in white mice encountered in any of our field studies.

Similar delayed occurrence in installed units was reported on a few other occasions, and in a very few instances infection appears to have been traceable to developed areas rather than to exposure in adjoining uncleared areas. McCulloch mentions 4 walking patients who contracted scrub typhus in an Australian hospital area that had been cleared and developed more than 20 months previously. Two similar instances involved surgical cases in an American hospital installed at Doba-

dura, New Guinea, for over a year. Nine cases, not traceable to outside exposure, occurred in a unit 3 to 4 months after moving into and clearing an area at Milne Bay. On Goodenough Island, the clearing of 2 American hospital areas resulted in exposure of some 100 personnel without immediate appearance of illness, but 48 infections developed, including cooks and office personnel, some 6 to 10 weeks after maximum exposure would have been expected.

More surprising are the urban and suburban cases in British-Indian troops reported by Parker. In a unit billeted in 1942 in a vacated college building with a grassy yard in the suburbs of Calcutta, there were 18 cases reported from 2 months to over a year after occupancy, while 6 cases occurred in another unit installed in the Alipore Botanical Gardens, and 3 in another unit camped on a golf course. Eleven cases occurred in Greek sailors billeted in this suburban area in 1941. Lusk reported 41 OXK positive cases from the environs of Calcutta in 1943, and Singapore also reported urban infection. Two units, bivouacked in war-derelict Burmese villages of Prome and Myitnge near Mandalay, developed 32 and 7 cases (including a clerk, a tailor, and a carpenter under minimum hazard), while other infections were traced to clearing the compound about the former living quarters of an abandoned tin-mining camp at Mawchi.

Abandoned native village sites were the loci of several military outbreaks of tsutsugamushi disease, including the severe Sansapor epidemic previously mentioned, and an outbreak in American troops on Bougainville reported by Anderson and Wing. A few other experiences in New Guinea and Northern Burma also led to suspicion of the vicinity of old Japanese food dumps, and of poor garbage disposal among early Chinese units in the early days along the Stilwell Road east of Ledo. Such conditions were believed to have caused a concentration of rats and consequently of mites with disaster to subsequent military visitors.

Island foci.—This effect of rat colonization upon foci was probably exaggerated on small, palm-covered Pacific Islands on which infection had become established. Bunamu Island, north of Bougainville, is a small (200×400 yards), coralline island with heavy vegetational cover, attracting hundreds of South Sea Island pigeons, which come there to feed, roost, and nest. These, and a dense population of rats (*R. browni*), were found to be heavily infested with *T. deliensis*. Three critical infections were contracted among a few Australian soldiers visiting the Island to hunt. Ponam Island, a small, coral atoll 2.5×0.5 miles, lying north of the Admiralty Group, was developed as a U. S. Naval base. A few natives were living on the Island, plus a few pigs and chickens. The rat population was not heavy. Mites were present. Twenty-one cases of infection with 2 fatalities developed among our Naval personnel within 4 or 5 weeks of occupation.

South Bat Island is an isolated, 46-acre island in the Purdy Group south of the Admiralties, on which the Australians and Americans established observation posts. Within a month and a half, 26 of 41 personnel had to be evacuated with scrub typhus and the Island was abandoned. We revisited the Island for 4 days about 2 months later in anti-mite treated clothing and recovered 3 virulent strains from pools of mites off local rats, which subsequently killed laboratory mice from one to two days average shorter time than any strains previously handled. This was the first definite recovery of natural infection in *T. deliensis*. There was obviously a saturated population of rats, and a correspondingly heavy infestation of *T. deliensis*.

Only 3 of 12 mite-pools were not invalidated by intercurrent infection, but since all 3 provided strains of *R. tsutsugamushi,* an intense endemicity was concluded to be present in the local mites. The abundance of hosts, with only internecine competition, permitting a rapid turnover of the rat-mite-rat cycle, supplemented by transovarial passage of the agent, appeared to provide ideal conditions for hyperendemicity. The high virulence of resulting laboratory strains may or may not have been chargeable to this rapid turnover. The Island was uninhabited, though it was visited periodically by natives to harvest the coconuts. An Australian team later visited the Island for several months, further demonstrating the protection afforded by treated clothing.

Owi Island in the Schouten Group was about 1 by 3 miles in size with a few native inhabitants, and had a mixed animal population of 2 kinds of rats and a marsupial bandicoot for mite hosts. Nearly two-thirds of the cases in the major Owi-Biak epidemic in the American Army occurred on this small Island.

The Maldives and Diego Garcia south of Ceylon, as well as the Andomans and Nicobar Island north of Sumatra, all provided cases in British Army and Navy personnel, and appeared to have a similar epidemiologic picture.

A number of coastal foci on New Guinea, and on Luzon and Samar had comparable coconut plantation environments, which provided ideal rat cover in the undergrowth due to neglect enforced by Japanese occupation of the region during the war.

Grass associations.—In the early days of the fighting in Papua and British New Guinea, a high proportion of the cases was contracted by units maneuvering or bivouacked in so-called kunai grass—a tall, rank grass reaching over a man's head in height when mature. No ecologic explanation was elicited for the fields and slopes bordered naturally by sharp, climax jungle margins. These margins were particularly hazardous in focal areas as determined not only by traceable case occurrence, but by rat trapping and mite sampling. Overemphasis, because of this early experience, caught some seasoned units by surprise when they encountered major epidemics under the coconut canopy of the beaches at Owi and on the coralline ridges of Biak Islands.

Similar rank grassy areas continued to furnish outbreaks in Dutch New Guinea. on the Philippine Islands, where it was called "kogan" and "talahib grass" respectively, and on Ceylon it was known as "illuk." In Malaya the same grass, called "lalang," has long been a source of cases. These fields are chiefly composed of one or both of two species, *Saccharum spontaneum* and *Imperator* spp. Both are found in the river margin foci of Japan. They may occur as volunteer, primitive fields, or as secondary invaders in abandoned, man-cleared areas. Under primitive methods of cultivation in Burma, India, and Ceylon, where the land is abandoned after 2 or 3 crops are harvested because of the influx of weeds, or because of progressive infertility as in Sumatra, the practice is commonly referred to as "cheena cultivation." Such fields provide attractive rat harborage, and are especially hazardous for military development. In consideration of the importance of these areas in Burma, Audy explored the possibilities of aerial mapping of potential "cheena" foci ahead of military envelopment.

There are reasonable grounds, therefore, to consider that no small part of the tsutsugamushi problem in the Asiatic-Pacific region is indirectly man-made and

to some extent man-spread. Cheena cultivation, abandoned village and garden sites, neglected coconut plantations, all encouraging native rat harborages are his doing—and military undoing. However, the domestic or commensal rats, so important in endemic typhus, are not generally the important mite hosts in scrub typhus, and it appears probable to the writer that isolated island foci may build up in the local rat and mite faunae following initial introduction of the etiologic agent by birds.

It is probable that it would take many faunal generations and some years for development of hyperendemicity in a new locality judging from other biological experience. For this reason and because of the difficulties that would have been encountered in strain maintenance, we felt safe in suggesting the absurdity of the notion that the Japanese intentionally or unintentionally were spreading the disease in occupied areas. This belief was frequently encountered among combat units, both verbally and in classified reports during the campaigns in both theaters.

Localization.—A peculiarity of tsutsugamushi disease re-emphasized all during the war was the observed localization of infection within larger endemic zones. Adjoining units established under practically identical conditions with relation to jungle margins in the same kunai or cheena field, or to coconut and scrub cover as a part of a local, continuous ecologic association, often showed striking "spottiness" in incidence. The impression was often as though a nidus of infection had just been initiated in the area but had not had time to envelope the entire area. This patchy distribution was reported by various investigators during the Australian, British-Indian, and American campaigns. It was known to the Japanese farmers to whom I talked in the long established foci along the Mongami and Agano Rivers. Two or three examples may be cited. Coastal fighting on Mindoro during reoccupation of the Philippines was through the grass-invaded, abandoned cane fields with nearly a hundred resultant cases. Five of these occurred in one-half of a split squad, the other half of which remained infection-free, though deployed approximately 200 to 300 yards away at all times during the first ten days when infection must have been contracted by the others. The previously mentioned outdoor theater area at Finchheaven is another instance of extreme localization. Sayers describes the 10-day location in jungle exercise of a detachment of about 100 officers and men along a stream in Burma, the officers' area being some 30 to 40 yards from that of the "other ranks." No cases occurred in the latter group with many more exposures, while 7 were contracted in the smaller officers' area. A certain ridge above the Kabaw Valley in Burma was dubbed "mite hill" because of the infection there of over 100 British soldiers during a 3-weeks' patrol duty of 2 companies. The rest of the battalion patrolling adjacent areas remained relatively free.

It is probable that such localization is a reflection of restricted movement within the local environment of mite-bearing rodents, permitting much slower spread of the infection than might at first be thought.

Seasonal occurrence. In Japan and Northern Formosa, the disease is highly seasonal for obvious reasons. In the Pacific and North Burma operations, no seasonal fluctuation was detectable and peaks of incidence appeared to be related to exposure during combat and exercise activities, as pointed out by Mackie et al. On the other hand, Audy and his colleagues have summarized evidence among

British-India troops before the envelopment of Central Burma which they feel could only have been a reflection of seasonal variation in mite activity and not solely a matter of troop exposure. The dry season in the focal parts of Eastern India, Ceylon, the Maldives, and Southern Burma is associated with a subsidence of case incidence not attributable to decreased operative functions. There are many variables in attempted correlation with seasons in the fluctuating exposure of mobile troops that could be misleading as compared to incidence in a static, civil population.

Most experience indicated that outbreaks occurred in troops during the initial development of an infected area (Table 2), while continuing sporadic cases after establishment of such a base (e.g., Dobadura in New Guinea) usually resulted from jungle exercises during staging rather than originating in the "civilized" area itself, The mites appeared extremely sensitive to the lowered humidity in cleared areas, and did not remain long on grass stems and objects above the damp ground litter, seeking a host.

The Mindoro, P. I., outbreak mentioned above was a surprising exception of mite prevalence under apparently adverse conditions. The ground at the base of the kogan grass was extremely dry and friable, and according to local report the area was in a second year of relative drouth. Yet 95 cases occurred in paratroopers fighting through the grass in localized spots, and *T. deliensis* was not uncommon in the ears of local rats from relatively dry burrows.

Few infections were attributable without question to the deep primary jungle itself, usually because at least some exposure to marginal cheena or beach areas had occurred during the incubation period. A few cases probably were contracted in the early fighting along the Stilwell Road in North Burma where clearing had not previously occurred and the climax rain forest was almost continuous. Survey of rats by Mohr and by Griffiths at Sansapor revealed heavier infestations of *T. deliensis* in the deep, shaded jungle or uncleared areas than in the more open habitats, and the American team in North Burma also caught *deliensis*-infested rats in the heavy rain forest. Cases in British troops in the Irrawaddy Valley in late 1944 "occurred between Mawlu and Naba, in a type of jungle which could hardly be called 'scrub.' It varied between tall trees meeting overhead and a certain amount of undergrowth, to thick impenetrable jungle as was encountered at Pinwe" (Audy). No previous native colonization was in evidence in the main line of advance in this section.

There was some evidence that infection followed along the drainage system in Southern Burma, but that the Indo-Burmese mountain range, and the Arakan Yomas range acted as "ecologic barriers" to the overlapping of rat populations, and hence to the eastward and coastward spread of the endemic areas. Under such circumstances it is difficult to visualize birds as important "carriers" of the infection in those sections. This is even more puzzling in Japan itself, where endemic areas have remained discrete and not spread into the foothills, at least along the margins of the upper cultivated fields.

It seems strange to the writer, with all the field work that has been done in Japan, that more information of the distribution of mites and mite hosts outside the endemic areas has not been reported, at least in accessible reports. Some of their literature indicates the disease is self-limited to the distribution of *T. akamushi,* while there is

meagre reference to what was regarded as the same species found in the ears of voles by Okumura in Korea and below Tokyo near Fujiyama. If these were actually *akamushi,* it is remarkable the disease has remained confined to the classic foci, since it has spread so readily to isolated islands in the Southwest Pacific and Indian Ocean. The epidemiology in Japan still offers fascinating and potentially profitable problems for investigation. It is unfortunate so little time could be spent in the field during our occupation.

Audy has called attention to the seasonal and local correlation between distribution of the disease and of *T. deliensis* in Burma, analogous to the observations of Nagayo and others on the coincidence of the disease and of *T. akamushi* in Japan.

In consideration of the over-all epidemiologic picture and the basic triad—(a) mites, (b) rodent (or avian) hosts, and (c) *Rickettsia tsutsugamushi*—it appears that the disease may become adapted to rather divergent local circumstances so long as the disease cycle is maintained. By early 1944, it became obvious due to the observed ecologic variations in foci that there was no such thing as a "typical scrub typhus environment." Surveys for localization of infection in indigenous fauna will probably continue to follow rather than precede human incidence as the chief means of detecting its distribution geographically. One of the most peculiar epidemiologic adaptations has occurred under restricted conditions of farming in the Pescadores (Boko) Islands. Coral rock-lined fences about the yards and gardens have provided good rat harborages and more intimate family contact with the mite vectors. Hence 70 per cent of the yearly incidence is in children under 15 years of age, and over half of these are under 5. It is probable that many of the adults in these Islands are immune. It is not surprising, then, that a low over-all death rate should be reported, though Kawamura is often quoted regarding the mild Pescadores strains which he even used in fever treatment of paresis patients.

Adaptation of the infection to semi-urban conditions at Calcutta, Mandalay, Kulewa, Rangoon, and Singapore, also attests to the necessity for revamping our ideas of its strictly rural or "scrub" character, while its high altitude occurrence in Northern India is another deviation of portent.

CONTROL OF TSUTSUGAMUSHI DISEASE

The early Japanese farmers attempted to prevent attacks of the minute akamushi or tsutsu-gamushi by wearing mite-tight clothing, using wrap leggings and garments with as few seams as possible, and often with a hood-like extension over the head. Similar reasoning regarding clothing for exclusion of mites was repeatedly embodied in unit and theater directives during the war regarding the wearing of leggings, or of trouser legs tucked inside sox and high boots, and of sleeved shirts when exposed in dangerous areas. However, such precautions presented at most a delaying, not an impassable barrier when not fortified with acaricides.

Actually, experience by extensive field observation indicated that personnel continuously on the move through mite-infested terrain, even where the abundant scrub-itch species abounded, were attacked in proportion to their loitering and resting in contact with the ground en route. The mites, occurring in, not above, the ground litter, were not brushed off the vegetation in passing, like ticks, nor was there the danger at first believed in casual contacts with stumps and rotting logs.

The lack of adequate information on the habits of the vector mites not infre-

quently resulted in the issuance of useless directives. Bathing was only dangerous where the clothing could not be laid on the open beach, and in tropical warfare, bathing is important to the health of the men. Judgment was not always exercised in restriction of this beneficial function for presumed local scrub typhus prevention. Pets, particularly dogs, often accompanied units with or without sanction, and naturally elicited concern as mite gatherers in the bush. However, in a given locality we did not feel they were a hazard to the men even if mites did attach to them (mostly in protected parts of the body) since they would not leave the animal until engorged, and would seldom find conditions favorable for continuation of another generation in the developed areas. On the other hand, the directive of the Surgeon of the Fifth Air Force on Owi Island to prevent rapid air transfer of potentially mite-infested dogs from this focus to advance areas such as the Philippines was unquestionably sagacious. Unit company officers at Sansapor were charged by directive with responsibility of having all dogs dusted with DDT powder, especially around the ears and anus, at least every 3 days, and in another directive it was stated that "mosquito repellent may be used on the legs and in the crotch" in addition to its use in clothing treatment. Men who tried this, never repeated application to the crotch regardless of directives. On Ponam Island, loose pigs belonging to natives were blamed for maintaining infection brought in by Japanese invaders and so were rounded up and penned, to the consternation of natives and pigs alike.

The over-all result, however, was beneficial by supplemental indoctrination of troops regarding prevention. Attractive posters were prepared in many areas to stimulate preventive practices. The biology on these was sometimes bad, showing mites waiting on grass stems, or the wrong species of "vector" illustrated by the uninformed draftsman.

Because the hoped-for vaccine from either chick-egg, mouse-, rat-, or cotton-rat-lung sources failed to materialize for field use, control of scrub typhus in military operations was predicated upon 2 basic principles, namely, temporary personal protection from mite attack with acaricides, and the more durable manipulation of the environment to produce unfavorable conditions for mite prevalence in staging and bivouac areas.

In advance operations, such as jungle patrolling, beach head landings, and staging exercises, exposure was unavoidable, and prevention was only possible through personal measures, chiefly use of GI insecticides applied to uniforms. Madden and his associates of the Department of Agriculture Laboratory in Orlando, Florida, reported in 1944 the value of insect repellents for protection against North American chiggers. McCulloch then adapted this method to needs of the Australian Forces, using a strictly anti-mite fluid, dibutyl phthalate, which proved to be one of the most durable for clothing treatment. The Australians achieved remarkable success in indoctrinating their troops regarding hand-application of the fluid to uniforms in formation, as evidenced according to McCulloch in a marked drop in their case rates by midyear of 1944. Protection was achieved in known endemic areas such as Ramu Valley during operations in which environmental control alone could hardly have been responsible.

The Americans in New Guinea adopted techniques developed by Bushland of the U.S.A.T.C. for dipping uniforms in a dimethyl phthalate-soap emulsion, but

too late to do more than aid in control of the Owi-Biak and Sansapor epidemics. In the latter, protection was afforded certain units according to Griffiths, and in a focus on Bougainville reported by Welt. Field demonstrations at Hollandia showed the superiority of dibutyl phthalate and benzyl benzoate in resisting cold laundering in the field, so that now these substances, plus an emulsifier, comprise the Army anti-mite fluid in quartermaster supplies. Machine methods were developed by the Chemical Warfare Service in the Philippines to impregnate uniforms before issue in anticipation of the final push to Japan. If this could be adapted economically to civilian needs in endemic areas, it would be a boon to further development of the tropics and of marginal lands in Japan. It is surprising that this method was so little used in the Burma campaign.

Consideration was given to DDT anti-mite dusting by airplane in advance operations, but it was shown by Bushland that only poor and temporary area control of mites was achieved even by more thorough hand operations on the ground than could be achieved from the air. Clean camp sites proved most efficacious in reducing the mite hazard, and the rapid tapering off of admissions as areas were developed even in the face of epidemics was very obvious. New cases continued longer in one bomb squadron on Owi Island, whose area was cleared by hand, than in an immediately adjoining squadron area rapidly stripped and cleared by heavy machinery.

It can be anticipated that cheena cultivation, and the redevelopment of tropical areas that had fallen into disuse during the war will continue to provide outbreaks, particularly among native laborers. Whether Army methods of control, particularly use of expensive insecticides, can be utilized feasibly remains to be seen.[5]

THE PRICE OF INVESTIGATION

It is fitting here to memorialize those laboratory and field workers who accepted the hazards and paid full price for investigating this treacherous disease agent during the war period. The record of the rickettsioses in the laboratory is a bad one, and tsutsugamushi disease takes its place as a worthy member of the group in this respect.

Dora Lush, Walter and Eliza Hall Institute, Melbourne, Victoria, Australia, died 20 May 1943.

Philip LeRoy Jones, Rocky Mountain Laboratory, Hamilton, Montana, died 28 September 1944.

Dr. Richard G. Henderson, National Institute of Health, Washington, D. C., died 20 October 1944.

David J. Hein, Lederle Laboratories, Pearl River, New York, died 22 October 1945.

Captain Jewel E. Roberts, MC, U. S. Army, Pathologist, 14th Evacuation Hospital, Assam, India, onset on plane en route home 3 August 1945, died in U.S.A. 16 August 1945.

[5] Since the cessation of hostilities the Orlando investigators have demonstrated the efficacy of area control of North American chiggers with benzene hexachloride (12 per cent gamma isomer) applied at the rate of 4 pounds per acre as a spray or dust. Elimination of mites for a month at least was shown.

Smadel and his associates at the Army Medical School also have found considerable promise in the protection afforded laboratory animals for the use of chloromycetin as a chemoprophylactic agent against this disease. If this discovery is confirmed under field trial, it could be of considerable importance in the absence as yet of an effective vaccine.

Four of these contracted infection through laboratory accidents, the last performed a necropsy on a patient who died of scrub typhus at an Army Evacuation Hospital near Ledo a few days before his departure. Dora Lush, earlier, and another technician, had previously sustained endemic typhus infections while engaged in preparation of cotton-rat-lung vaccine at the National Institute for Medical Research in England, thus confirming the lack of residual, non-specific immunity between these 2 diseases.

Mr. Jones had previously contracted two other insidious laboratory infections—tularemia and lymphocytic choriomeningitis.

Additional, non-fatal infections occurred in personnel at the following institutions or field stations: Assistant in Heaslip's laboratory near Adelaide, South Australia, February, 1940; field officer (A.G.G.) of the U.S.A. Typhus Commission in Assam, December, 1943, and two others of the British Scrub Typhus team at Imphal, India, October, 1945; technicians at the National Institute of Health, October, 1944, at the Rocky Mountain Laboratory, June, 1945, at the Naval Medical Research Institute, Bethesda, Maryland, July, 1945, at the Lederle Laboratories, October, 1945, and at the Army Medical School, Washington, D. C., January, 1947.

In seven of these eleven non-fatal cases, vaccination had previously been done, but in view of the antigenic heterogeneity of strains previously discussed, it is difficult to assess the value of the vaccine, especially since therapeutic medication of various sorts was also instituted during hospitalization. As with typhus and spotted fever, symptoms developed in spite of vaccination and 4 of these provided strains of *R. tsutsugamushi* by blood passage to mice.

The sources of infection were, in two instances, accidental penetration of needles into hands in attempted recovery of falling syringes containing mouse-passage material, wound in finger through a rubber glove by broken end of a contaminated pipette, rat-bite on finger during passage of cotton-rat-lung strain, contamination of abrasions on fingers in 4 cases, and, in one each, possible contamination of conjunctivae of eyes and droplet inhalation. Three cases in field laboratories were possibly bitten by mites; the sources of the remaining infections are unknown.

SUMMARY OF MAJOR CONTRIBUTIONS INCIDENTAL TO RECENT HOSTILITIES WHICH WILL BE OF LASTING BENEFIT TO SCIENCE AND THE DEVELOPMENT OF THE TROPICS

1. Expedited knowledge both intensively and extensively of the distribution of focal areas in the Asiatic-Pacific Region, and provided laboratory confirmation of the identity of the disease in these areas.
2. Contributed following information on vectors:
   (a) Confirmation of natural infection in larval *Trombicula akamushi*.
   (b) Provided first proof of natural infection in *T. deliensis*.
   (c) Provided unequivocal evidence of transovarial and transstadial transmission of the agent through various stages of *T. deliensis*.
   (d) Added biological data on both species in nature, including post-larval stages of *T. deliensis* for important comparative studies.
   (e) Added evidence of the close taxonomic relationship of these two species, and improved rearing methods for experimental studies of chigger mites.
3. Augmented by almost a third (and perhaps a half when all are described)

the known species of trombiculid mites, and contributed several species and varieties of animals, particularly rodents, new to science.

4. Contributed important data on rodent and avian hosts of the vectors in various localities, and their environmental and geographic distribution.

5. Added 5 species of rats, one field mouse, and one insectivore to the list of proved naturally infected mite hosts and placed additional suspicion on the epidemiologic role of birds.

6. Stimulated refinement of laboratory techniques and development of specific complement-fixing tests, together with discovery of antigenic differences in strains of the etiologic agent.

7. Improved the practices necessary for environmental control, and provided information on more durable acaricides for personal protection by treatment of clothing.

8. Greatly intensified the search for effective vaccines and for therapeutic drugs.

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The following references were freely drawn upon in the above discussion as well as several unpublished military reports and documents (most of them classified), which it is not appropriate to list for this nontechnical presentation. Many of the latter have been documented by the writer in "A History of Scrub Typhus and Scrub Itch in the American Army in World War II," Historical Division, Office of the Surgeon General of the Department of the Army. To Colonel J. H. McNinch and Miss Sylvia Gottwerth of that Division, and to General S. Bayne-Jones, formerly Director of the U.S.A.T.C., the writer is indebted for use of data in their respective files. Data on incidence in various forces were obtained through courtesy of Colonels T. F. Whayne, C. H. Sams, and Lt. Colonel C. Z. Berry of the U. S. Army, Captains J. J. Sapero and R. L. Ware of the U. S. Navy, and of Drs. E. V. Krogh, I. M. Mackerras, and R. J. McCulloch formerly of the Australian Land Forces.

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Supplemental note: Since preparation of the above lecture, Dr. J. R. Audy kindly provided the writer in Malaya with the translation of an important additional war-time report by Lt. Col. Kiyosi Hayakawa, of the Japanese Army Medical Corps, dated 13 February 1946, summarizing data published by several medical officers in Japanese journals in 1944 and 1945, together with unpublished information. Reference is made to outbreaks in Japanese Forces or European internees in Burma, Thailand, Malaya, Sumatra, and Java. The agent causing milder infections in these areas compared to Japan, he named *Rickettsia orientalis* var. *tropica* n. sp., which, even if valid, is antedated by Amaral and Monteiro. Strains were reportedly recovered from patients, mites, and rats in various areas. Central and Southern Thailand may therefore be added to the map, but his data on incidence are insufficient to be included in the comparative table. C.B.P.

FURTHER STUDIES ON DUODENAL MUCUS AS A FACTOR IN AGE RESISTANCE OF CHICKENS TO PARASITISM[1, 2, 3]

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Much literature has appeared during the last 20 years on the immunity elicited by animals to metazoan parasites. At present, investigators in this field agree that helminths are capable of initiating immune reactions much the same as other recognized infectious agents. Comprehensive reviews of the work on immunity to helminths may be found in the monograph by Taliaferro (1929) which includes all important references up to that date, in the compilation by Culbertson (1938) of the literature from 1929 to 1938, and in the contributions of Lapage (1937), Taliaferro and Sarles (1939), and Ackert (1942).

Of the different types of immunity elicited by animals to helminths one of the most important is age resistance. This does not arise as a result of previous or contemporary infection but develops as the host grows up to approximate sexual maturity. Younger animals either lack the defense mechanism possessed by older ones or they provide more suitable conditions for the development of the parasite. Those hosts which become infected during the susceptible phase of their life are capable of expelling most of their helminths when the resistant age is reached; and when hosts reach a resistant age before becoming infected, the helminths' development is retarded. This is particularly true of the large roundworm of fowls, *Ascaridia galli.*

The habitat of this nematode is the anterior portion of the small intestine, particularly, in a section beginning a few centimeters posterior to the entrance of the bile ducts. In an inquiry into possible structural differences in this portion of the intestine of young and older fowls, Ackert and Edgar (1938) made histological studies of the worm habitat in chickens ranging from two to 320 days of age. The most striking structural difference observed was an increase in numbers of goblet cells in the epithelial lining of the intestine. The goblet cells per area increased from 2.9 in 2-day chicks to 10.7 in 124-day chickens after which no further increases were found in chickens up to 320 days old.

These increases in numbers of goblet cells followed closely the retardations of worm growth due to age of fowl as found by Herrick (1926). With the aid of the present senior author, duodenal mucus from resistant chickens was introduced into a nutrient medium prepared by Ackert, Todd and Tanner (1938) in which were cultured immature *A. galli.* Results of several tests indicated that the mucus from resistant chickens contained some factor which retarded the growth of the worms (Ackert, Edgar and Frick, 1939a, 1939b), while that from young chickens 66 days old largely lacked the resistance factor (Ackert, 1942).

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[1] Contribution No. 253 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Science, Manhattan, Kansas.

[2] Portion of a thesis presented by Lyman P. Frick as partial fulfillment of the requirements for the degree Doctor of Philosophy in Parasitology at Kansas State College.

[3] Appreciation is expressed to Dr. L. D. Bushnell, Department of Bacteriology, for counsel and materials and to Dr. H. H. Laude of the Kansas Agricultural Experiment Station, for aid in making statistical analyses of data.

Following these preliminary studies, a number of additional tests have been run in efforts to determine the rôle played by goblet cells and duodenal mucus in the phenomenon of age resistance of chickens to *A. galli*. Descriptions of these tests and the results follow.

MATERIALS AND METHODS

All the fowls were obtained as day-old White Leghorn chicks given rations considered to be adequate and raised indoors under carefully controlled conditions. The worms were obtained from the chickens which at 14 to 21 days of age were fed about 500 *A. galli* eggs.

Recovery of the Mucus

All samples of the mucus used in this problem were collected in essentially the same way. Immediately after the chicken was killed, the body was opened and the portion of the small intestine between the point of entrance of the bile ducts and the yolk sac diverticulum was removed. The mucus was obtained by slitting the intestine on the side opposite the mesenteric connection and then gently scraping the mucosa with a clean, blunt spatula. The process of scraping was done as rapidly as possible to prevent overlong exposure to air and to lessen the opportunity for autolysis. The whole procedure of mucus recovery was completed in from six to nine minutes. Any debris or undigested food material in the lumen was removed.

Since the mucus was used differently in each of the several tests within each series of experiments, the specific treatment of it will be described as each series is considered. Reference is made to "young mucus" and "old mucus" to indicate mucus from young and old chickens, respectively.

Recovery of the Worms

All worms were recovered in the same way. After the intestine was slit, the worms were floated off into sterile saline solution which had been kept at a temperature corresponding to the body temperature of the host, that is, 106–108° F. The worms were allowed to wash approximately an hour before being used. They were transferred with a hooked needle, and care was taken to handle them as little and as carefully as possible. These precautions were necessary to avoid removal of the protective mucous covering of the worm, for if this occurred, the worms became extremely susceptible to mold and fungous attacks.

Culture Media

The original medium used by Ackert, Todd, and Tanner (1938) in the artificial culture of *Ascaridia galli* had the following formula:

NaCl	2.25 gm
$CaCl_2$	0.06 gm
KCl	0.10 gm
$NaHCO_3$	0.04 gm
Dextrose	0.62 gm
H_2O	1000 cc

This solution was used over a base of cornmeal agar; but in the present work it was found that this base made the culture extremely favorable for molds and fungi,

and that it could be omitted. The above medium was used in all of the preliminary tests, and in the comparative study on autoclaved mucus.

Although this medium as used by Ackert, Todd, and Tanner gave very good results, it had certain disadvantages, one of which was that the formula had to be diluted with four volumes of distilled water before use. Since the original total ionic concentration of the medium had not been calculated, the arbitrary addition of water without subsequent check tests would give only a rough estimate of the isotonicity of the medium for *A. galli.*

In 1939, Fenwick reported on partial results obtained in his studies on the artificial culture of *Ascaris* larvae. The solution used by Fenwick included the following:

NaCl	0.80%
$CaCl_2$	0.02%
KCl	0.02%
$MgCl_2$	0.01%

This solution, which had a total ionic concentration equivalent to a 0.832 per cent saline solution, Fenwick found by a series of tests to be isotonic for *Ascaris* larvae. He also found that the larvae could maintain themselves in this medium with dextrose added in concentrations up to 0.1 per cent, provided that the concentration of NaCl was adjusted to keep the total ionic concentration at 0.832 per cent.

Since larvae of *Ascaris suum* and *Ascaridia galli* are rather similar, tests were run in which Fenwick's solution was substituted for the original medium. The solution was tested with concentrations of dextrose of 0.0155 per cent, 0.050 per cent, and 0.100 per cent. In each case the concentration of NaCl was adjusted so that the total ionic concentration remained at 0.832 per cent. As nearly as could be determined, the above concentration was also isotonic for *A. galli.* While each of these three concentrations of dextrose gave good results, on the average, the addition of dextrose to 0.05 per cent gave the best results.

The original pH of the above formula was 7.0, which was well within the optimum range for the parasite. The addition of mucus to the solution often necessitated an adjustment of the pH. All pH determinations were run on hydroquinone and glass electrode potentiometers.

Ackert, Todd, and Tanner used as a check on their nutrient solution a 0.9 per cent saline solution. This solution was also used in all preliminary tests; but in later tests, Fenwick's base solution without the addition of dextrose was used.

Measurement of the Parasites

The original procedure for the measurement of the worms consisted in magnifying them six diameters by the use of a photographic bellows, and then tracing the outline of the worm on onion-skin paper. The length could then be found by measuring the outline with a calibrated wheel. Previous to this work, this procedure was used in all work in which linear measurements were involved.

This method had several serious disadvantages for measurement of live worms. The worm was often exposed to room temperatures for several minutes; it was subjected to drying; it was often handled too much; and, moreover, it was extremely difficult to trace the outline of the active worm. Consequently, for the present

work, a modification of the former method was developed. The worms were first photographed against a non-reflecting background on 35-mm film. A six-diameter enlargement of the photograph was made on onion-skin paper and the outline traced. Then, by multiplying this length by the reducing power of the camera, which, with the lens system used, was calculated to be 3.33, the actual length of the worm could be found. Advantages of this method were that the worm was not subject to drying, cold, or unnecessary handling, and that the measurements were extremely rapid and accurate. After death the nematodes were measured directly by the use of the photographic bellows.

EXPERIMENTAL DATA

The principal function of the goblet cells is the secretion of mucus, which serves in part as a lubricant for the intestine. Mucin, probably the most important constituent of mucus, is a glyco-protein generally considered to be a factor in the prevention of auto-digestion of the mucosa.

Comparative Study with Autoclaved Mucus

To determine *in vitro* effects of mucus on the intestinal worm, *A. galli,* some preliminary tests were run. The technique was as follows: A 90-day chicken was

TABLE 1.—*Results of tests for an inhibitory nematode growth factor in duodenal mucus from chickens three months old. (From Ackert, Edgar, and Frick, 1939a)*

Culture media	Larvae in culture days	Average gain or loss in length (mm)	Per cent gain
Experiment I			
Isotonic-salt-dextrose solution plus mucus*	3.0	2.2	4.0
Isotonic-salt-dextrose solution (control)	2.5	11.8	18.5
Isotonic-salt solution (no nutriment)	2.0	−2.5	0.0
Experiment II			
Isotonic-salt-dextrose solution plus mucus	3.0	8.5	12.8
Isotonic-salt-dextrose solution (control)	3.0	27.0	36.9
Isotonic-salt solution (no nutriment)	3.0	−0.2	0.0
Experiment III			
Isotonic-salt-dextrose solution plus mucus	3.0	2.1	2.9
Isotonic-salt-dextrose solution (control)	3.0	14.3	19.8
Isotonic-salt solution (no nutriment)	4.0	−11.0	0.0

* The pH of the isotonic-salt-dextrose solution was 6.1; that of the autoclaved mucus was 6.0.

killed and the mucus collected and autoclaved for 30 minutes at 25 pounds of pressure. The autoclaved material was then added to the worm culture. The ratio in each case between mucus and nutrient medium was 3:65. From three trials, results were obtained as shown in Table 1. These data indicated that while the worms in the nutrient solution were able to increase their length from 18.5 to 36.9 per cent, the worms in the cultures to which mucus had been added, even though in the presence of nutriment, grew only 2.9 to 12.8 per cent. From the results of these trials it appeared that duodenal mucus from resistant chickens contained some factor which was unfavorable for the growth of *A. galli.*

Even though the addition of 90-day mucus to the culture had inhibited the growth of the worms, there was no definite proof that this inhibition might not have been due to some mechanical reaction resembling crenation, brought about by a change in the concentration of the medium with the addition of the mucus. In order to check this possibility, another test was run in exactly the same manner as the previous tests except that in this case mucus from a 45-day chick was used. The results of this test in which the worms grew as well in the presence of the mucus as in its absence, indicated that mucus from 45-day chickens lacked the power to retard the growth of the worms (Table 2). Since mucus from 90-day chickens appeared to have an inhibitory power against the worms, and mucus from 45-day chickens lacked this ability, it seemed desirable to test mucus from chickens of various ages, especially that from chickens ranging from 45 days to three or four months of age. From several such tests the results, which are shown in Table 2, indicated that there was a progressive increase in the potency of the mucus as an inhibitor as the age of the chicken increased. For example, the worms in Group B in the presence of mucus from 54-day-old chickens gained an average of 17.91 mm

TABLE 2.—*Effect of autoclaved mucus from different aged chickens on* A. galli *cultured in vitro*

Worm group	Age of mucus (days)	Experimental worms	Controls
		Average gain or loss (mm)	Average gain (mm)
A	45	9.59	9.20
B	54	17.91	21.74
C	63	14.40	17.09
D	66	13.55	15.88
E	70	12.09	42.38
F	107	4.75	28.53
G	117	−0.83	8.25
H	125	−4.49	9.02

as compared with 21.74 mm for the control worms without mucus; and in Group H the worms in the presence of mucus from 125-day chickens failed to gain and even lost 4.49 mm in length while the controls in the absence of the mucus gained 9.02 mm. From the results of these tests it is seen that beginning with mucus from chickens 54 days of age, the growth of the worms was retarded progressively to the age of at least 125 days.

Comparative Study with Unautoclaved Mucus

The results of the tests described thus far have been indicative, but not conclusive, primarily because the numbers of samples used were not large enough to obtain a good estimate. Consequently, a much larger experiment was so planned and arranged as to make it possible to test biometrically the growth of worms between the ages of 30 and 90 days in mucus media from chickens 60 to 140 days of age.

The procedure in this experiment was somewhat different from that of the preceding tests. Heretofore, the mucus was autoclaved before being used. The fact that inhibition occurred in autoclaved mucus indicated that the inhibitor is not of the nature of an antibody. However, it seemed desirable to run tests with both autoclaved and unautoclaved mucus, so that one series of tests might serve as a check on the other. In all tests in the experiment under consideration the mucus was used in the untreated (unautoclaved) condition.

The cultures were made very much as they were in the experiments on autoclaved mucus except for one major difference. Certain preliminary trails showed that it was possible to culture a number of worms in one container provided that each had a sufficient volume of solution. A goal of ten worms was set for each lot, but that number was not always available. Seven to ten worms were used in three-fourths of the 41 lots and an average of five worms in the remaining lots.

Since all the factors in the experiment were controlled except the mucus samples, it was necessary to run only one series of controls. This series was run early in the experiment on worms between 30 and 90 days of age, taken by 10-day intervals. When feasible, each group in the series was matched by a group of worms cultured in the isotonic, non-nutrient medium. The data which are given in Table 3 show that all worms (controls) in the nutrient medium grew in length, whereas those (checks) in the non-nutrient medium lost length.

The medium used in all tests of the experiment was the one based upon Fenwick's solution which was described above. The chickens which were used as a source of

TABLE 3.—*Control and check series of* A. galli *cultured in vitro for three days. Control worms cultured in nutrient medium; checks in non-nutrient medium*

Worm group	Age of worms (days)	Controls (nutrient medium)			Checks (non-nutrient medium)		
		Mean init. length (mm)	Mean final length (mm)	Mean gain (mm)	Mean init. length (mm)	Mean final length (mm)	Mean gain or loss (mm)
A_1	30	38.29	49.56	11.27	40.85	39.87	−0.98
A_2	30	47.29	62.81	15.51	....	....	....
B_1	40	41.40	45.83	4.42	41.30	37.83	−3.47
B_2	40	55.71	63.99	8.28	....	....	....
C	50	66.66	74.10	7.44	61.21	59.63	−1.57
D	60	81.74	102.71	20.96	61.10	53.44	−7.65
E	70	81.47	89.40	7.92	....	....	....
F_1	80	90.56	100.21	9.65	....	....	....
F_2*	80	62.57	67.73	5.15	....	....	....
F_3*	80	60.96	68.88	7.91	....	....	....
G_1	90	92.35	107.66	15.31	....	....	....
G_2*	90	66.76	71.64	4.88	....	....	....
G_3*	90	71.08	75.58	4.49	....	....	....

* Males only.

worms were divided into seven main groups, some of which were divided into two or three sub-groups. When feasible, the worms from one sub-group were cultured in mucus from the other; the worms from that sub-group in turn were cultured in mucus from the first. In all cases, the mucus was obtained from white leghorn chickens raised under controlled conditions.

If any effects were to be produced by the mucus they would show in at least three days; therefore the cultures were run for this length of time, after which the worms were killed in hot A.F.A. solution and the various measurements made and recorded. The complete data of this phase of the experiment were so extensive that they are presented in general summary.

A survey of the means of the increases or losses in length of worms indicated that a 20-day age basis of mucus would be suitable for statistical comparisons. The general summary of the data is given in Table 4 and the outline of the analysis of variance for these data in Table 5 which shows that the differences of the growth of *A. galli* in the younger age groups of mucus and the oldest age group were highly significant. The data gave an F value of 6.82 which surpassed the 1 per cent level of significance, 4.57, for 3 and 28 degrees of freedom.

TABLE 4.—*Summary of retardation of worm growth; data on per-worm basis of* A. galli *cultured in untreated mucus from chickens of varying ages*

Age of worm (days)	Trial	Age groups of mucus (days) A 60–80	B 80–100	C 100–120	D 120–140	Sums
30	1	−0.468*	0.9856	−1.461	−1.574	−2.5174
	2	−0.834	−0.9350	−1.165	−0.361	−3.2950
	Sum	−1.302	0.0506	−2.626	−1.935	−5.8124
40	1	0.1030	−0.173	−0.9156	−0.714	−1.6996
	2	−0.4602	−1.603	−0.1397	−0.531	−2.7339
	Sum	−0.3572	−1.776	−1.0553	−1.245	−4.4335
50	1	−0.0008	−1.4261	−0.6766	−2.9303	−5.0338
	2	0.3636	−0.9981	−0.5820	−0.1870	−1.4035
	Sum	0.3628	−2.4242	−1.2586	−3.1173	−6.4373
60	1	0.610	−0.3454	−0.3912	−1.5270	−1.6536
	2	0.808	−2.8212	−2.8076	−1.6954	−6.5162
	Sum	1.418	−3.1666	−3.1988	−3.2224	−8.1698
70	1	−0.9360	0.4430	−1.4921	−0.8577	−2.8428
	2	0.3595	−0.3726	−1.5300	−2.5899	−4.1330
	Sum	−0.5765	0.0704	−3.0221	−3.4476	−6.9758
80	1	−0.7511	−0.1641	−0.392	−2.2370	−3.5442
	2	−2.3630	−0.1813	0.729	−3.9514	−5.7667
	Sum	−3.1141	−0.3454	0.337	−6.1884	−9.3109
90	1	−0.7525	−1.8480	−0.0892	−2.9670	−5.6567
	2	−1.9920	−0.6476	−0.8578	−6.9690	−10.4664
	Sum	−2.7445	−2.4956	−0.9470	−0.9360	−16.1231
	Sums	−6.3135	−10.0868	−11.7708	−29.0917	−57.2628
	Group means	−0.902	−1.441	−1.681	−4.156	.....

* Millimeters.

Thus far a highly significant difference in worm-growth inhibition has been shown to exist between groups of worms cultured in different ages of mucus involved in this experiment. In order to determine where this difference lies a *t* test was applied to the group means, using data derived from the analysis of variance. This test showed that while there was not a significant difference in retardation of worm growth between the 60–80 and 80–100-day groups, the difference between the 60–80 and the 100–120-day group closely approached the 5 per cent level of significance, and the difference between the 120–140-day group and any other group was highly significant. Referring to Table 4, it will be seen that the group means are as follows: Group A, −0.902; Group B, −1.441; Group C, −1.681; and Group D, −4.156. For the 5 per cent level of significance, a difference of 0.805 was necessary, and for the 1 per cent level, 1.09. Thus it is seen that the difference between Group C and Group A closely approached significance and the difference in inhibiting power of Group D (oldest) and any other group was highly significant.

Mucus Extraction

The results of tests made with both autoclaved and unautoclaved whole mucus, introduced into cultures of the worms, have shown conclusively that duodenal mucus from resistant chickens contains some factor which may inhibit the growth of *A. galli*.

TABLE 5.—*Analysis of variance for effects of duodenal mucus of varying ages on* A galli

Source of variation	Degrees of freedom	Sum of squares	Mean square
Total	55	97.04	...
Worms	6	11.06	1.84
Mucus	3	21.91	7.30**
Worm-mucus interaction	18	34.08	1.89
Error	28	29.99	1.07

** Highly significant: $F = 7.30/1.07 = 6.82$ surpasses the 1 per cent point, 4.57 for 3 and 28 degrees of freedom.

This duodenal mucus is a heterogeneous mixture of mucin and other chemical constituents, as well as varying amounts of partially digested food material, and undoubtedly certain tissue fragments.

The next step in the study was to attempt to determine in which portion of the mucus the active principle is contained.

A test was therefore set up in which mucus from birds 171 days of age was recovered and washed with two 30-cc portions of sterile saline. The mixture was centrifuged between each washing, and the supernatant liquids saved. The washed insoluble portion was ground and extracted with 60 cc of sterile saline. The washings and the extract were added to the nutrient solution in the usual ratio of 3:65, the suspensions autoclaved, the pH adjusted, and the worms added.

The mixtures were autoclaved for two reasons: first, the results obtained with autoclaved material in the comparative study were almost identical with the conclusive results obtained with the untreated mucus; and second, maintaining the sterility of the unautoclaved mucus entails a great deal of effort. Thus the efficiency of the experiment could be increased by autoclaving the mucus before use.

TABLE 6.—A. galli *cultured in 171-day autoclaved mucus washings and mucus extract*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain or loss (mm)
11D1	Iso-salt	6	66.30	77.00	10.70
11D2	plus	6	56.10	60.00	3.90
11D3	dextrose	6½	68.00	75.00	7.00
11D4		6	71.40	74.00	2.60
				Ave. gain	6.05
11W1	Nutrient	9	76.50	99.25	12.75
11W2	plus mucus	8	73.10	67.66	− 5.44
11W3	washings	9	64.60	62.50	− 2.10
11W4		8	73.64	64.00	− 9.64
				Ave. loss	− 1.11
11G1	Nutrient plus	8	66.30	59.50	− 6.80
11G3	ground	8	73.10	59.50	− 13.60
11G4	mucus	6½	57.80	63.50	6.70
	extract			Ave. loss	− 4.57

The results of this test, which are shown in Table 6, indicated that the inhibitory factor exists in either of two phases, one of which is soluble in 0.832 per cent saline, and the other insoluble; or that it is entirely soluble and only appeared in the extract because it was not entirely leached out of the solid fraction. The worms cultured in the presence of the washings exhibited an average loss of 1.11 mm, while those in the extract lost 4.57 mm. This would indicate that the fraction of the inhibitor present in the extract is the more potent. The control worms gained an average of 6.05 mm.

A second test was run on a slightly different basis. Two 115-day-old chickens were killed and approximately 12 cc of mucus recovered. The mucus was added to 240 cc of the nutrient medium and the mixture allowed to stand nine days at refrigerator temperature. This procedure, it was hoped, would determine whether the factor was entirely contained in the soluble fraction of the mucus, or whether part of it was contained in the insoluble portion. During the nine days the mixture was left standing, any soluble substances should be leached out.

After the nine-day interval, the mixture was centrifuged and the supernatant liquid decanted. On the basis of a previous trial run with mucus from 97-day-old

chickens, it was found that of 5.0 cc of raw mucus recovered, 4.0 cc were soluble and 1.0 cc insoluble. In the case now under consideration the ratio between soluble and insoluble fractions was doubled in order to allow for the presence of debris in the mucus. On the basis of both this ratio and the quantity of liquid recovered, the soluble portion of the mucus was calculated to be in a concentration of 1 : 35.

Due to loss in transfer, only 2.0 cc of the calculated 4.8 cc volume of solids were recovered. The insoluble material was added to 75 cc of the nutrient solution, making a dilution of 1 : 38.

Five 70-day worms of approximately the same size were measured and put into the 75 cc of the 1 : 38 dilution of solids and 75 cc of the 1 : 35 dilution of washings, respectively. The cultures were allowed to run three days, after which the worms were killed in hot A.F.A. solution, and remeasured.

The results of this test were quite different from those of the preceding tests of the same type. In the test under consideration, the worms in the 1 : 38 dilution of solids showed an average gain of 1.482 mm per worm, a gain approximately equal to what would be expected from 70-day worms; whereas the worms in the 1 : 35 dilution of the soluble fraction showed an average loss of 1.48 mm per worm. Thus, the gain in one was equal to the loss in the other.

TABLE 7.—*Titration of unautoclaved mucus washings*

Dilution	Loss of length of worms (mm)	Gain in length of worms (mm)
1– 35	7.40	...
1– 70	2.49	...
1– 140	1.13	...
1– 280	2.99	...
1– 560	...	1.46
1–1120	...	1.59
1–2240	...	4.52

The inference from this test is that the growth-inhibiting factor is contained only in the soluble fraction of the mucus. It is highly improbable that the portion of the insoluble material that was lost could have contained much of the inhibitor, for the mucus-medium mixture was thoroughly shaken twice a day during the period of washing.

Titration of the Washings

The experiment described above gave evidence that the inhibitory factor is entirely contained in the soluble fraction of the mucus.

In order to determine the effect that dilution might have on the efficiency of the inhibitor, a titration was run with dilutions ranging from 1 : 35 to 1 : 2240. Four 80-day worms were cultured in each of the dilutions. The results of this test are shown in Table 7 and Fig. 1.

It will be seen from the results of this experiment that the efficiency of the inhibitor is almost directly dependent upon concentration.

Effect of Filtration on Washings and Extract

In the preceding tests, it has been shown that the growth inhibitor contained in duodenal mucus taken from resistant chickens which had been previously parasitized is contained in the portion of the mucus soluble in 0.832 per cent saline solution.

Following these tests an experiment was run in which the washings and the extract of mucus taken from resistant chickens which had not been parasitized were filtered before use.

This test was designed to show two things: first, the effect that mucus from unparasitized birds might have on the worms; and, second, the effect that filtration might have on the washings and the extract.

The technique of mucus recovery, washing, and extraction in this test was the

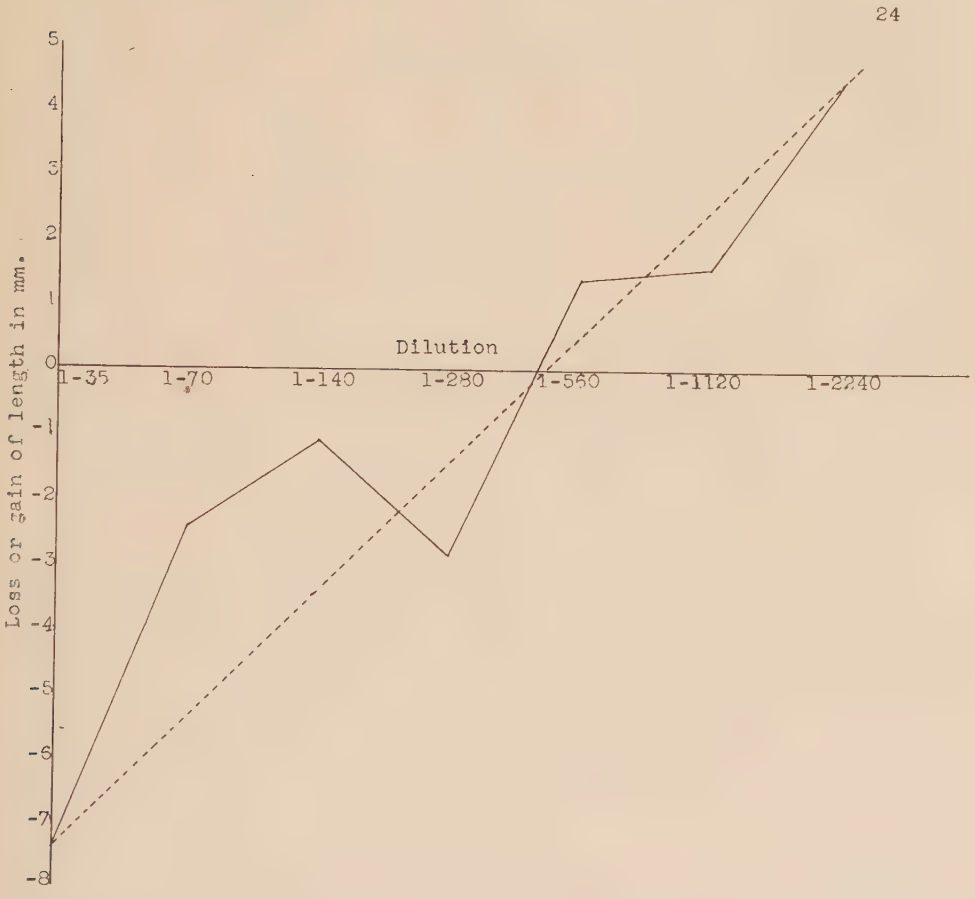

FIG. 1. Titration of mucus washings. Mucus not autoclaved. Age of mucus, 115 days; worms, 80 days. – – – – – – – – Theoretical growth curve which would be given if the effectiveness of the washings were directly dependent upon concentration. ———— Titration curve.

same as in the previous test on extraction. After extraction, the extract and the washings were filtered through a Berkefeld candle.

The data on this test are shown in Table 8. In general, the results were quite comparable to those of the first test on extraction. In addition they indicated that mucus from unparasitized chickens of the age ordinarily considered resistant also contained the inhibitory factor, and furthermore, that the factor was filterable.

This test indicated, as did the first test of this series, that part of the factor is contained in the insoluble fraction of the mucus. However, the second test of this series gave evidence that the inhibitory ability is contained only in the soluble frac-

TABLE 8.—*Effect of filtration on washings and extract of mucus from 90-day unparasitized chickens*

No.	Culture	Days in	Length in (mm)	Length out (mm)	Gain or loss (mm)
13D1	Regular nutrient medium	4	65.32	73.00	7.68
13D2		3	62.00	79.50	17.50
13D3		5	64.00	57.66	− 6.34
				Ave. gain	6.28
13G1	Nutrient plus ppt. from filtered ground mucus	3	76.00	70.00	− 6.00
13G2		3	68.00	62.00	− 6.00
13G3		3	78.64	74.00	− 4.64
				Ave. loss	− 5.55
13F1	Nutrient plus filtrate from filtered mucus extract	5	78.00	84.66	6.66
13F2		3	66.00	76.00	10.00
13F3		4	80.00	104.50	24.50
13F4		4	90.00	90.00	0.00
				Ave. gain	10.29
13W1	Nutrient plus filtered mucus washings	5	48.00	40.66	− 7.34
13W2		4	58.00	50.50	− 7.50
13W3		3	60.00	60.00	0.00
13W4		3	46.00	52.00	6.00
				Ave. loss	− 2.21

tion. In neither of the two cases in which two phases of the inhibitor were evident was the mucus allowed to stand long enough to complete the leaching process.

Recovery Tests

In all the tests considered thus far, the effect on the worms of mucus from resistant fowls had been manifested in the inhibition of growth of the worms. This fact suggested that the inhibitory factor in the mucus interfered in some way with the normal nutritional processes of the worm. However, it was not known whether the effects exerted by the factor were of a permanent or temporary nature.

In order to determine this point, a test was run in which worms, after being subjected to the actions of mucus for a certain period of time, were remeasured and transferred to the regular nutrient solution. The results of this test are shown in Table 9, Trial 1.

From these data it will be seen that the nematodes, after being cultured eight to nine days in the presence of the factor, had, with one exception, the ability to recover and resume growth when transferred from the mucus cultures to the nutrient

TABLE 9.—*Recovery of worms after transfer from mucus cultures to nutrient medium*

Culture no.	Days in mucus culture	Gain or loss in mucus culture (mm)	Length out of mucus culture (mm)	Days in nutrient culture	Length out of nutrient culture (mm)	Gain or loss in nutrient culture (mm)
Trial 1						
11G1	8	− 6.80	59.50	5½	77.00	17.50
11G3	8	−13.60	59.50	6	81.00	21.50
11W1	9	12.75	89.25	4	85.00	− 4.25
Trial 2						
13G1	3	− 6.00	70.00	6	88.50	18.50
13G2	3	− 6.00	62.00	2	62.50	0.50
13G3	3	− 4.64	74.00	1	79.50	5.50

solution. In each of the cases where recovery occurred, the worms were able to make up any loss of length resulting from the mucus treatment, and in addition, to add extra growth. The loss of length after being in the 11W1 culture may have been due to some mechanical injury to the worm, or it may have been an expression of the inherent variability of the worms. The nematodes used in this test were part of those used in the experiment on extraction.

This experiment was repeated on some of the worms used in the test on the effects of filtration. These results are shown in Table 9, Trial 2. These data were very much like those of the previous test. In the mucus cultures the worms suffered an average loss of 5.55 mm. After transfer the worms gained an average of 8.17 mm which made up the loss incurred in the mucus culture plus a gain over the original length.

These tests indicate that the effects exerted by the inhibitory factor on the worms are nutritional in nature, and are temporary.

DISCUSSION

The increased resistance or immunity of older fowls to the large roundworm, *Ascaridia galli,* probably is due to more than one factor. Graham, Ackert, and Jones (1932) obtained some evidence of an acquired resistance of chickens to this nematode by previous infections from small numbers of *A. galli* eggs. Eisenbrandt and Ackert (1940) found increased resistance in fowls to this nematode following mild immunization in three of nine groups of chickens, and Sadun (1947) has recently announced what appears to be quite conclusive evidence of acquired immunity in fowls to *A. galli* from feeding large numbers of the nematode eggs. Kahn (1936) and others found that older or mature animals show far greater capability for tissue-antigen reactions than do young ones. In nature, part of the control of such nematodes as *A. galli* must be attributed to acquired host immunity.

Another factor in control which develops with age in the absence of worm antigens is termed age resistance, or increased natural resistance. Application of this principle to worm control in commercial poultry production, that is by raising young chickens for 10 or more weeks on clean ground or in battery cages before exposing the fowls to worm eggs, has greatly reduced the *Ascaridia* worm problem. The elucidation of this resistance factor has been the goal in this study.

As the anterior portion of the small intestine is the habitat of *A. galli,* portions of it from young and older chickens were studied histologically by Ackert and Edgar (1938) with the result of finding increased numbers of goblet cells per area in the older fowls. Accordingly, more mucus was observed in the intestinal lumens as the chickens became older. When such mucus was autoclaved and introduced into nutrient nematode media, the growth of immature *A. galli* was retarded (Ackert, Edgar, and Frick, 1939a), whereas, such mucus from young chickens 66 days old largely lacked the inhibitory factor (Ackert, 1942).

That the inhibition is not due wholly to the larger volume of mucus from the older fowls is shown by a tabular comparison (Table 10) of goblet cell counts with the group sums (Table 4) of the worm-growth inhibitions resulting from treatment with mucus from chickens of comparable ages. It showed that the results of the present experiment can be partially correlated for chickens from 58 to 124 days of age with the goblet-cell counts, since numbers of goblet cells and increase in nematode

growth inhibition go hand in hand. Beyond the age of 124 days, however, the goblet cells do not increase, while the inhibitory factor markedly increases. The results of this and other comparisons point to the presence of an inhibiting factor in the mucus of older chickens. The larger volume of mucus in the older fowls is a secondary factor.

Among the findings from the various tests presented above are: that the inhibiting factor is present in both autoclaved and unautoclaved duodenal mucus of older fowls; that it may be dissolved from the mucus in 0.832 per cent saline; that it is nutritional in character and may be temporary in effect. This last phase of the study supports in part the view of Chandler (1935, 1936) that the prime factor in the immunity of rats to *Nippostrongylus muris* is local, a property of the intestinal mucosa, that it serves to inhibit normal worm nutrition and that the nematodes may resume growth and development in the absence of the factor. That *A. galli* normally feeds on host ingesta (which would include mucus) was shown by Ackert, Whitlock, and Freeman (1940).

TABLE 10.—*Lack of correlation between goblet cell count per area and inhibition of nematode growth resulting from treatment with mucus from chickens of comparable ages*

Age of chicken days	Number of goblet cells per area	Worm growth inhibition group sum
58	8.7	− 6.3135
71	9.3	−10.0868
124	10.7	−11.7708
131	9.2	−29.0917

A new technique was developed during the present study for handling the mucus. At first, the use of unautoclaved mucus entailed a great deal of extra work. It was extremely difficult to prevent lysis of the mucus, and it was impossible to recover the mucus in a sterile condition due to the presence of great numbers of bacteria in the intestine. However, it was discovered that if the mucus is allowed to soak about nine days in 0.832 per cent saline, all of the inhibitor-containing fraction will go into solution. This washing or leaching can be carried out at low temperatures which will reduce bacterial action to a minimum; and, following leaching, the mixture can be filtered to remove all microorganisms. The bacteria-free filtrate may then be used as were the whole mucus-medium mixtures.

SUMMARY

Further studies on duodenal mucus as a factor in age resistance of chickens to the intestinal nematode, *Ascaridia galli,* are here summarized.

1. Additional evidence is presented which shows that immature *Ascaridia galli* will grow in an isotonic salt-dextrose medium, but will not do so in an isotonic non-nutrient medium.

2. Larger volumes of mucus from more numerous duodenal and intestinal goblet cells occur in older than in younger fowls.

3. Evidence supported by statistical analysis is offered to show that duodenal mucus from old, resistant chickens differs qualitatively from mucus of young susceptible chickens. This difference in mucus of different ages is manifested by retarding effects of old mucus on the growth of *A. galli* when added in constant pro-

portions to *in vitro* cultures of the worm, and by the lack of such nematode growth retardation in young mucus.

4. While the efficiency of the mucus from older chickens as a growth inhibitor, and the increase in number of goblet cells per unit area of intestine as the chick grows older, are undoubtedly associated, they cannot be perfectly correlated. Little difference occurs in numbers of goblet cells per area in 58-, 71-, 124- and 131-day-old chickens, whereas the inhibitory factor increases markedly in effectiveness during these intervals.

5. Tests with a constant amount of mucus have shown that the efficiency of the mucus as a worm growth inhibitor varied directly with the age of fowl from which the mucus was taken.

6. Extraction and filtration of the mucus have shown that the inhibitory factor is entirely contained in the portion of the mucus which is soluble in 0.832 per cent saline.

7. A titration of the mucus has indicated that the effectiveness of the inhibitory factor is directly dependent upon concentration.

8. The inhibitory factor appears not to depend on previous or current infection. A number of tests indicate that this factor in duodenal mucus is not of the nature and character of an antibody, since mucus which had been autoclaved at 15 pounds pressure for 20 minutes remained as effective as untreated mucus. Autoclaving at this pressure, which is equivalent to a temperature of 125° C, is known to destroy antibodies.

9. Evidence is offered which indicates that the effects exerted on the worm by the inhibitory factor are nutritional and temporary in nature.

10. During this study, certain new techniques were developed for problems of this nature. These include a method for the measurement of the living worms, a nematode *in vitro* culture medium, and a technique for handling unautoclaved duodenal mucus.

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A CONTRIBUTION TO THE STUDY OF CERTAIN AVIAN STRIGEIDS (TREMATODA)

Asa C. Chandler and Robert Rausch*

The examination of more than 200 species of birds by one of us (R.R.) during the past few years, has disclosed a number of interesting strigeids, some of which are considered in the present paper. Three species appear to be undescribed and two of these are of particular interest in that they occur in passeriform birds, a group rarely parasitized by strigeids. In addition to the descriptions of the new species, remarks concerning *Hysteromorpha triloba* (Rud. 1819) are included.

Diplostomum crassum n. sp.
(Fig. 3)

Diagnosis: Body 1.38 to 1.5 mm long. Forebody short and thick, bent dorsally, and measuring about 0.35 to 0.5 mm along the dorsal side, and 0.55 to 0.8 mm along the ventral side, with a dorso-ventral diameter of 375 to 520 μ. Hindbody 835 μ to 1.0 mm long, with a dorso-ventral diameter of 44 to 58 μ. Oral sucker 85 to 100 μ in diameter, and 85 μ long, flanked by two deep pseudosuckers. Pharynx smaller, about 75 to 80 μ in diameter, and 85 μ long. Ventral sucker not seen, presumably concealed under the dense vitellaria in forebody. Holdfast organ, as seen in lateral view, thick, tongue-like, and about 290 μ long; its anterior border situated about 285 μ from anterior end. Ovary situated just behind junction of forebody with hindbody, measuring 70 to 100 μ in length, and 160 to 175 μ dorso-ventrally. Anterior testis situated just behind ovary, 200 to 235 μ long, and 250 to 285 μ dorso-ventrally. Posterior testis, as seen in lateral view, slightly overlapping anterior one and the larger of the two, measuring 220 to 330 μ in length, and 310 to 350 μ dorso-ventrally. Posterior border of hind testis 240 to 316 μ from posterior end of body. Vitellaria very dense in forebody, extending forward almost to pharynx, but not entering holdfast organ; in hindbody, dense follicles extend to near posterior end of body, ventral to reproductive glands. Eggs up to about 25 in number, found throughout length of hindbody; they measure 100 to 105 μ by 61 to 63 μ.

Host: Quiscalus versicolor Vieill. (bronze grackle).

Habitat: Small intestine.

Locality: Near Garland, Manitoba; collected September 5, 1947.

Type: U. S. Nat. Mus. Helm. Coll. No. 46340.

This species differs markedly from other members of the genus *Diplostomum* in the thickness of the forebody, which gives it more the appearance of a *Strigea.* Unfortunately, only three specimens, taken from a single host, were available for study; all were mounted laterally, or nearly so. The study of a large series of specimens, with sections, may make it necessary to erect a new genus for this peculiar parasite. It is the only member of the genus *Diplostomum* to be recorded from a passeriform bird.

Uvulifer erraticus n. sp.
(Fig. 1)

Diagnosis: Body 1.08 to 1.55 mm long. Forebody thin, curved ventrally at posterior end, 415 to 462 μ long, and 164 to 193 μ broad. Hindbody club-shaped, 638 μ to 1.1 mm long, very narrow (62 to 110 μ) at region of attachment to forebody; maximum diameter 193 to 264 μ. Oral sucker shallow, saucer-shaped, with margin unevenly curled backward; diameter 97 to 110 μ, and length only 24 to 35 μ. Pharynx immediately behind the oral sucker, about 45 μ in diameter. Ventral sucker apparently absent, or very inconspicuous. Holdfast organ in posterior third of forebody, about 88 to 90 μ long; approximately round, or slightly oval. Ovary round, situated

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about 2/5 to 1/3 length of hindbody behind the junction with forebody; 70 to 77 μ in diameter. Testes tandem, immediately behind ovary in dorsal part of body; all three contiguous. Testes round or slightly deeper dorso-ventrally; about 150 to 160 μ in diameter. Vitellaria scattered throughout posterior portion of hindbody, extending forward to a point slightly anterior to ovary. Genital cone protrusible, showing a bulb-like terminal expansion in one specimen. Eggs few, a maximum of three seen in a single specimen; 96 to 103 μ by 58 to 61 μ.

Host: *Toxostoma rufum* (L.) (brown thrasher).
Habitat: Small intestine.
Locality: Greenville, Ohio; collected April 21, 1945.
Type: U. S. Nat. Mus. Helm. Coll. No. 46341.

Since, in the specimens available, it is not possible to determine whether a "genital bulb" is present in the ventral wall of the bursa, the characteristic feature distinguishing the genus *Uvulifer* from *Pseudodiplostomum,* this species can only be tentatively placed in the former genus. It resembles species in the genus *Uvulifer* more than those in *Pseudodiplostomum* in the broad oral sucker, seen less conspicuously in *U. prosocotyle* (Lutz), and in *U. gracilis* Yamaguti, and in the poor development of the ventral sucker. A discussion of the "genital bulb" in the genus *Uvulifer* is given by Dubois and Rausch (1948).

Seven specimens of this parasite were obtained, all from a single bird. This host also harboured 4 specimens of *Plagiorhynchus formosus* Van Cleave, 1918.

The occurrence of this species in a passeriform bird is very unusual, since all other members of the genera *Uvulifer* and *Pseudodiplostomum* are found in kingfishers.

Neogogatea pandionis n. sp.

(Fig. 2)

Diagnosis: Length 1.00 to 1.47 mm. Forebody not sharply demarcated from the hindbody. Anterior part of body, to anterior border of holdfast organ, thin, slightly rolled ventrally at sides, and 350 to 570 μ long by 190 to 255 μ wide. Posterior portion of body approximately as wide as deep, constricted posteriorly into a narrow, truncated cone; maximum diameter 200 to 286 μ; cone 34 to 58 μ in diameter, and 60 to 150 μ long. Oral sucker not distinct in specimens at hand, obviously very small and poorly developed. Pharynx larger, 42 to 52 μ in diameter, its anterior border only about 25 μ from anterior end. Ventral sucker absent. Holdfast organ large and elongate, nearly as wide as body; posterior end not clearly defined.

Sex glands obscured by vitellaria in most specimens. Ovary small, about 66 μ in diameter, situated at one side of posterior portion of anterior testis. Testes longer than wide, and apparently about as deep as wide; length of anterior testis 110 to 120 μ long, and 75 to 96 μ deep. Posterior testis larger, about 120 μ in each direction in one specimen, and 167 μ wide and 105 μ long in another. Vitelline follicles large, confined to holdfast organ anteriorly, but extending beyond it posteriorly on either side, ventrally, to about level of middle of posterior testis. Cirrus pouch elongated; its proximal portion enlarged into a cylindrical, thin-walled seminal vesicle 155 to 175 μ long, then narrowing to an ill-defined tube in posterior part of body, about 88 to 130 μ long. Eggs 95 to 115 μ long, and 58 to 73 μ broad.

Host: *Pandion haliaëtus carolinensis* (Gm.) (osprey).
Habitat: Small intestine.
Locality: Waushara County, Wisconsin; collected April 13, 1947.
Type: U. S. Nat. Mus. Helm. Coll. No. 46342.

Two hundred and fifty-four specimens of this strigeid were obtained, along with 129 specimens of *Nematostrigea serpens* (Nitzsch), all from a single host.

The genus *Neogogatea* was established by Chandler and Rausch (1947) for a Cyathocotylid found in *Bubo v. virginianus* (Gm.). This second species possesses all the generic characters as given, but differs markedly in its smaller size, more elongate body, less obvious oral sucker, and smaller pharynx.

Hysteromorpha triloba (Rud. 1819)
(Figs. 4 and 5)

When we attempted to identify strigeids from the double-crested cormorant, *Phalacrocorax a. auritus* (Less.), it was found that they agreed in most respects with the published descriptions of *Hysteromorpha triloba* (Rud.), but differed in regard to the holdfast organ. This organ apparently has not been well described in the published descriptions. Through the kindness of Dr. Gustav Swanson, we were able to obtain the unpublished master's thesis of Hawkins (1932), in which the holdfast organ of *H. triloba* has been correctly figured.

This organ is described by Hawkins as follows: "The contracted state of the holdfast is very variable. In an extended condition it is funnel-shaped, measuring approximately 0.430 mm in diameter. In a contracted state the most prominent feature is the median furrow; the holdfast proper appearing as a thickening of the surrounding tissue." The appearance of the holdfast organ is shown in figures 4 and 5 of the present paper. The trilobed appearance of the anterior edge of this structure appears to be characteristic.

This strigeid appears to be a common parasite of the double-crested cormorant. A total of 1,602 specimens was taken from a bird collected at Horicon Marsh, Wisconsin, and infected cormorants were also collected at Delta, Manitoba.

A slide of our specimens has been deposited in the U. S. Nat. Mus. Helm. Coll., slide number 46343.

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Explanation of Plate

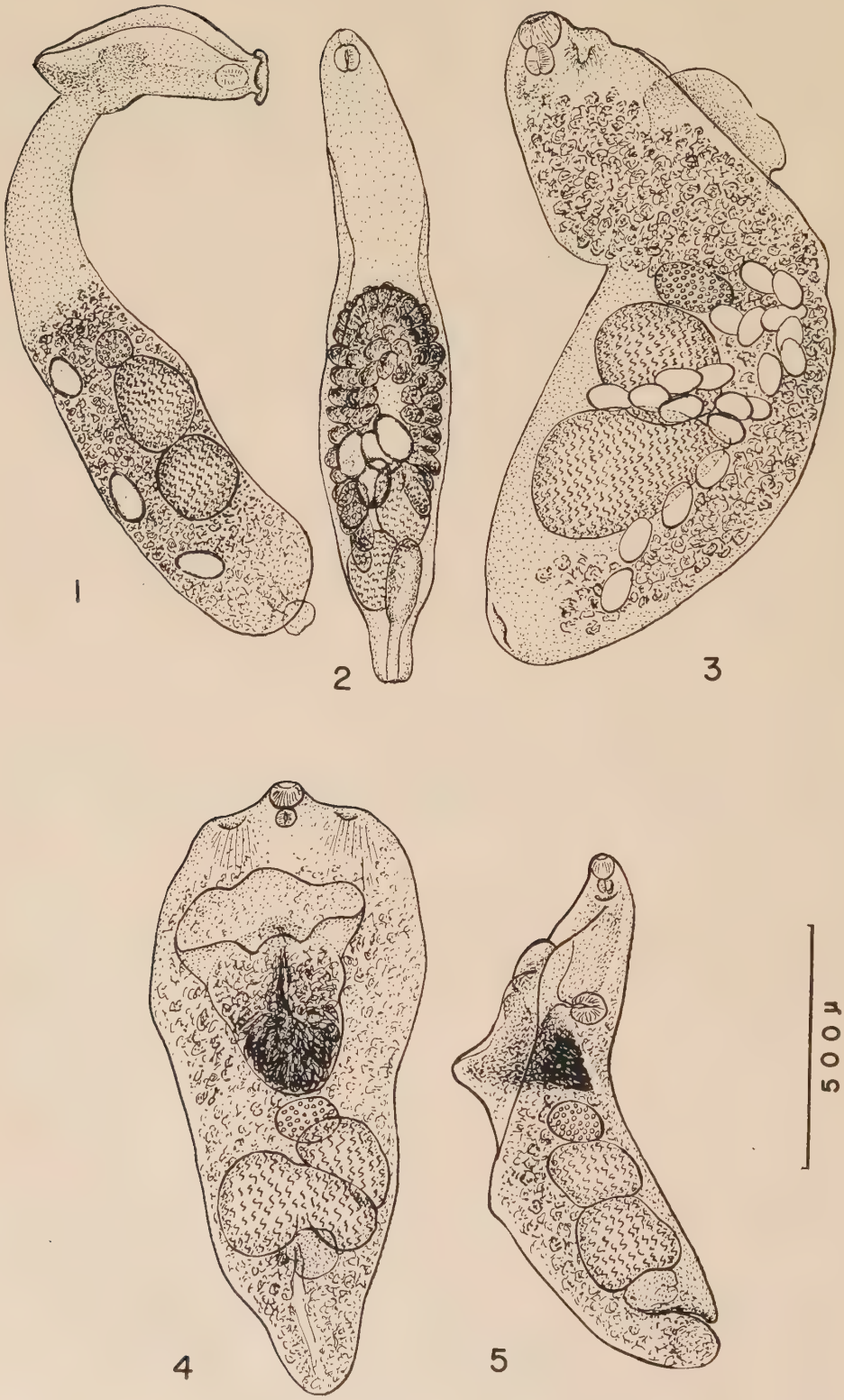
Fig. 1. *Uvulifer erraticus* n. sp., from *Toxostoma rufum* (L.).

Fig. 2. *Neogogatea pandionis* n. sp., from *Pandion haliaëtus carolinensis* (Gm.). Ventral view.

Fig. 3. *Diplostomum crassum* n. sp., from *Quiscalus versicolor* Vieill.

Fig. 4. *Hysteromorpha triloba* (Rud.), from *Phalacrocorax a. auritus* (Less.). Ventral view.

Fig. 5. *Hysteromorpha triloba* (Rud.), lateral view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PSEUDOPHYLLIDEAN CESTODES FROM ALASKAN PINNIPEDS

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Some thirty-three species of pseudophyllidean cestodes have been described from seals and sea-lions but the descriptions are so incomplete and imperfect that it is virtually impossible to recognize any of them with certainty. Redescriptions of species, on the basis of incorrectly determined specimens, have undoubtedly added to the confusion. In the absence of information concerning life cycles, consideration must be given to the possibility that representatives of a single species may complete their development in different host species and furthermore that, as a result of development in different hosts, individuals of the same species may manifest differences in size and shape, in rate of growth and sexual maturity, and in extent of development of various tissues and organs. Consequently, specific determination of specimens presents a particularly difficult problem. The pseudophyllidean cestodes of seals and sea-lions have been assigned to nine different genera, but the generic concepts are so indefinite and their limits are so poorly defined that there is no agreement concerning either the number of valid species or the generic groups to which they should be allocated. The uncertainty and confusion regarding the morphology and taxonomy of these cestodes were discussed by many earlier authors and more recently by Mueller (1937) and Wardle, McLeod and Stewart (1947). Most of the species have been referred, at one time or another, to the genus *Diphyllobothrium* Cobbold, 1858 as emended by Lühe (1910).

The genus *Diphyllobothrium,* as defined by Lühe, is a heterogeneous assemblage and taxonomic revision of these tapeworms was proposed by Mueller (1937) and by Wardle, McLeod and Stewart (1947). The latter authors stated: "It is a cumbersome group of about 70 species—many of them of dubious validity—and comprises forms from toothed-whales, seals, sea-lions, carnivorous land mammals and fish-eating birds. Several species have been recorded from humans and one even from a snake. It has always been an unsatisfactory genus to define and analyze, and particularly difficult to evaluate have been the forms from seals and sea-lions that have been recorded by numerous writers." Wardle et al erected a new genus, *Cordicephalus,* to contain the species found in seals and sea-lions and recognized four species: *Cordicephalus phocarus* (Fabricius, 1780); *C. tectus* (Linstow, 1892); *C. arctocephalinus* (Johnston, 1937); and *C. quadratus* (Linstow, 1892). All other species from seals and sea-lions were regarded as identical with one or other of the four accepted species. The remaining species of Lühe's genus were distributed among six other genera: *Diphyllobothrium* Cobbold, 1858; *Diplogonoporus* Loennberg, 1892; *Dibothriocephalus* Lühe, 1899; *Glandicephalus* Fuhrmann, 1921; *Adenocephalus* Nybelin, 1931; and *Spirometra* Mueller, 1937.

MATERIAL AND METHODS

The material utilized in the present study came from various sources and at different times. In an earlier report, Stunkard and Schoenborn (1936) described

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specimens from the bearded-seal, *Phoca barbata,* and referred them to the species, *Diphyllobothrium lanceolatum* (Krabbe, 1865). This material was collected in 1933, at St. Lawrence Island off the Alaskan coast, and submitted for identification by The American Museum of Natural History, New York. More recently, cestodes from the fur-seal, *Callorhinus ursinus,* and from the sea-lion, *Eumetopias jubata,* collected at St. Paul's Island, Alaska, July, 1945, by the U. S. Fish and Wildlife Service, were referred for identification by Dr. Victor B. Scheffer, of the University of Washington, Seattle. The hosts were identified by members of the U. S. Fish and Wildlife Service. The material consisted of scores of specimens from each of the two host species and provided adequate samples of the species represented. Grateful acknowledgment is made here to Dr. Scheffer for the privilege of studying this collection. In addition, the cestodes from fur-seals, reported by Stiles and Hassall (1899) as *Bothriocephalus sp.,* were loaned from the U. S. National Museum through the kindness of Dr. E. W. Price.

Representative specimens of the cestodes described in this paper were prepared as whole mounts, stained with either Ehrlich's haematoxylin or Mayer's paracarmine; others, previously stained to locate the sexually mature proglottids, were cut in serial sections in transverse, frontal and sagittal planes and stained with haematoxylin and erythrosin. Study has been concerned with anatomy as a basis for specific determination; often the fixation was not good enough for the preservation of finer histological structure. Since there is so much confusion and uncertainty concerning the morphology and specific identity of cestodes from seals and sea-lions, it appears necessary to present detailed descriptions of the present material.

DESCRIPTIONS

Species No. 1

These specimens, from *Phoca barbata,* taken at St. Lawrence Island, off the Alaskan coast, were described by Stunkard and Schoenborn (1936) as *Diphyllobothrium lanceolatum.* The gross morphology of the specimens was recorded, but fixation of the material was so poor that details, particularly of the excretory tubules, can not be traced. The parenchyma is so vacuolated that it is impossible to identify the excretory tubules with certainty. Moreover, the figure of a proglottid, given in the earlier paper, is a semi-diagrammatic optical section, dorsal to the genital atrium, and may give an erroneous impression of the copulatory organs and their relationships.

There is a single genital pore in each proglottid. It is situated near the middle of the ventral surface, at or near the posterior edge of the velum of the overlapping anterior proglottid. The pore is oval to slit-like, elongate in the anteroposterior direction and opens from a large genital atrium. The atrium extends about one-fourth of the length of the proglottid; it is deep, reaching to the medullary zone, so the cortical parenchyma is interrupted at this region. The cirrus sac opens into the anterior, the vagina into the central, and the uterus into the posterior portion of the atrium. To supplement the earlier description, figures of the entire worm, of a mature proglottid and of the reproductive organs are presented in the present paper (Plate I, Figs. 1–4).

Species No. 2

This species, from *Callorhinus ursinus,* is represented by many worms, none complete with terminal proglottid, and detached pieces of strobilas, usually 10–40 mm

in length. Most of the detached pieces are fully gravid. The specimens reported by Stiles and Hassall (1899) as *Bothriocephalus sp.*, and deposited in the U. S. National Museum, all belong to this species.

The scolex (Figs. 5–6) is pointed anteriorly, oval in dorsoventral aspect, and cordiform laterally; it measures 1.9–2.2 mm in length, 1.2–1.5 mm in width, and 1.6–2.0 mm in depth. The bothrial grooves extend the entire length of the organ and may be wider at either end, or wide at both ends with a narrower portion near the middle. Gland cells, similar to but less numerous than those described by Fuhrmann (1921) in *Glandicephalus antarcticus* (Baird, 1853), were observed in sections of the scolex.

The scolex is followed by a neck region, approximately one-half the length of the scolex, in which transverse furrows indicate the formation of young proglottids. The proglottids are always single, with no evidence of subdivision, and the reproductive systems are always single in each segment. Occasional fenestration of a proglottid was observed and apparently the strobila breaks easily at such weakened places (Fig. 9). There is a tendency for the older proglottids to separate from each other as the integrity of the strobila is weakened at the posterior end. The proglottids are craspedote, but the lateral margins are not markedly serrate. The neck is approximately 0.65 mm in width and the strobila gradually widens and the segments become longer. The longest worms measured about 120 mm in length with a maximum of 210 proglottids. The first genital rudiments are visible about 30 mm from the scolex; 30–40 mm later the first eggs appear in the uterus, but differences in degree of extension and contraction make such measurements of little significance. The following measurements in mm from 6 fairly well extended specimens are more significant.

Proglottid	*Length*	*Width*
1	0.06–0.072	0.7–0.85
50	0.18–0.23	1.2–1.5
75	0.20–0.45	1.6–2.0
100	0.45–0.60	2.0–2.60
140	0.70–1.5	2.0–3.50
160	1.30–1.75	3.0–4.0
200	1.70–3.5	4.5–6.0

The first genital rudiments appear in segments 70–80; the first eggs in segments 130–145; and the segments begin to detach at about segment 200. In a cross section 3.86 mm wide and 0.79 mm thick (Fig. 8), the cuticula is 0.006–0.010 mm thick; there is a very thin layer of external circular muscles; a row of longitudinal fibers; then the cortical cellular zone about 0.14 mm in thickness, the external portion of which contains longitudinal muscle fibers; then a vitelline zone, about 0.11 mm in thickness; then a loose parenchymatous zone, about 0.1 mm thick, in which there are scattered longitudinal muscle fibers; then a narrow band of transverse muscles, about 0.02 mm thick; then the medullary zone, 0.1–0.15 mm in thickness, in which the testes, ovary, and genital ducts are located. The total thickness, computed by adding the thickness of the several layers, is slightly more than the thickness of the proglottid, but this apparent discrepancy is explained by partial overlapping of the layers. There are very few dorsoventral muscle fibers.

The excretory system consists of cortical and medullary longitudinal canals which extend throughout the strobila. Whether they communicate with each other could not be determined with certainty, but there may be connections in the scolex and possibly there are other connections in the strobila. The cortical ducts are the more conspicuous, 12–20 on the dorsal side and a like number on the ventral side. On either side there are 8–12 larger vessels, 0.04–0.05 mm in diameter, which lie among the vitelline follicles, usually near the inner margin of the vitelline layer and other, usually smaller vessels, some of which are external to the vitellaria and others which extend into the medullary zone. The number of ducts is not constant; two of the larger longitudinal ducts may converge to form a single one, and one may divide to form two diverging canals which continue as parallel ducts. Moreover, in each proglottid, there are transverse tubules connecting all the major cortical longitudinal canals. Both the transverse and longitudinal ducts receive smaller tubules which branch and presumably lead to the flame cells. The variation in number and location of the cortical excretory ducts is so great that, contrary to the opinion of Zschokke (1903), no taxonomic significance is attached to them. In the medullary portion of the proglottid there are 2 longitudinal tubules, 0.015–0.025 mm in diameter, one on either side, mediad of the nerve, which have a slightly sinuous course. In sections, these ducts often appear to be double-walled, one tubule within another (see Talysin, 1930), and small

granules in or on the walls sometimes stain intensely with erythrosin. These medullary ducts receive smaller ducts at irregular and infrequent intervals. Whether they lead to flame cells or communicate with the cortical excretory ducts could not be determined. At the posterior end of each proglottid, or more often in the anterior end of the succeeding one, there is a very small transverse duct, 0.005–0.008 mm in diameter, which connects the longitudinal vessels of the two sides.

There are separate male, vaginal, and uterine pores which open to the surface of each proglottid. The male pore is median, ventral, about one-third the length of the proglottid from the anterior end. The cirrus sac extends dorsally and often anteriad and measures 0.18–0.25 mm in diameter. It is often flattened on the anterior side and consequently wider than long. Frequently there is a transverse lip which overhangs the anterior side of the pore. Usually there are 2–5 short transverse grooves, with shallow pits between them, in the median portion of the proglottid anterior to the male pore. They recall similar structures figured for *D. arctocephalinus* by Johnston (1937) and for *D. arctocephalini* by Drummond (1937). The cells are arranged radially around the pits and the condition is similar to that observed in young proglottids at the formation of the male genital pore. The cirrus is often partially extruded, but when retracted it forms a coiled tube within the cirrus sac. Near the pore the tube is straight 0.06–0.07 mm in diameter, with a lumen of about 0.015 mm, and is lined with cuticula. The coiled portion in the dorsal part of the cirrus sac is wider and lined with epithelium. A short duct leads from the cirrus sac to the seminal vesicle, a muscular-walled sac, about the same size as the diameter of the cirrus sac, which lies immediately dorsal and posterior to it. The cirrus sac and seminal vesicle occupy the medullary zone, extending across it. From the seminal vesicle a coiled, thick-walled duct extends posteriad for a short distance and then opens into a thin-walled vas deferens which passes posteriad in a sinuous course, dorsal to the uterine coils. Anterior to the ovary it breaks up into smaller ducts which subdivide in turn and form the vasa efferentia which lead to the testes. The opening of the seminal receptacle into the cirrus sac is at or near the level of the vaginal pore and the opening of the vas deferens into the seminal vesicle is at or near the level of the uterine pore. The testes are arranged in a single layer in the medullary portion of the proglottid and they are not delimited segmentally. They are spherical to oval, 0.04–0.09 mm in diameter, some 500–800 follicles in each proglottid, about 18–28 from anterior to posterior limits of a proglottid. They extend mediad as far as the ovary and uterine fields while in the anterior part of the proglottid they converge and follicles of the two sides may become continuous.

The vaginal pore is located 0.07–0.14 mm posterior to the male pore. It is usually oval, sometimes circular or sometimes a transverse slit. There is a short, narrow duct, usually sinuous, which passes dorsally and opens into an expanded, thin-walled portion of the vagina. This duct passes posteriad, in a sinuous course, ventral to the uterus and then dorsal to the ovary; here it expands into a retort-shaped seminal vesicle, similar to that of *D. mansonoides* as represented in Figure 18 by Mueller (1935). The uterine pore is slightly lateral, either right or left of the median plane and 0.15–0.36 mm posterior to the vaginal pore. Its lateral position is indicated in young proglottids (Fig. 7) before the genital ducts and pores are formed.

The vitelline follicles are oval, 0.012–0.055 mm in diameter; they surround the proglottid dorsally and ventrally in the cortical zone; follicles of the two sides are continuous at the anterior end of the proglottid but are interrupted posteriorly in the median field. The vitellaria are not delimited segmentally, but form a continuous sheet in successive proglottids. Vitelline ducts pass mediad, posteriad, and ventrad; at the level of the oötype, ducts of the two sides unite to form a small vitelline receptacle.

The ovary is ventral, near the posterior end of the proglottid, and consists of two lateral wing-like expansions connected by a narrow median portion. Transversely it measures 0.5–0.9 mm. The oviduct arises from the median, posterior portion. There is a distinct oöcapt, after which the duct is constricted to a narrow tube. It then widens, winds posteriad, receives a narrow duct from the seminal receptacle, turns and receives a larger duct from the vitelline receptacle, continues posteriad and ventrad whence it turns dorsad and enters Mehlis' gland where it expands to form the oötype. Mehlis' gland is flattened anteriorly, hemispherical posteriorly, and measures 0.28–0.36 mm in transverse diameter. The initial portion of the uterus coils in Mehlis' gland but the course is not the same in different proglottids; there are 2 or 3 coils on each side above the ovary which contain somewhat spherical eggs with thin shells,and a series of loops extends from one side to the other as the uterus passes forward. The number of loops is variable, usually 5–6 on each side anterior to the ovary. The course is irregular and much variation was noted in different proglottids. The course of the uterus can be traced only in young proglottids (Fig. 10), where the eggs are few and tend to lodge at the ends of the loops; in fully gravid segments (Fig. 9) the masses of eggs in superimposed limbs prevent the identification of individual loops and give the uterus a "rosette" appearance. The terminal leg of the last loop is usually enlarged to form an inflated sac, from which the duct leads to the uterine

pore. Mature eggs measure 0.05–0.062 by 0.037–0.044 mm. They have thick shells, are operculate, and are usually somewhat flattened on one side, but this condition may be the result of fixation.

Species No. 3

Material of this second species from *Callorhinus ursinus* consists of many young worms complete with terminal proglottids, older complete and incomplete ones with gravid proglottids, and pieces of strobilas.

The scolex (Figs. 11–12) is subglobular, oval to ovate in dorsoventral aspect, usually wider anteriorly, almost circular in lateral aspect; 1.1–1.69 mm long, 0.9–1.3 mm wide, and 1.2–1.9 mm thick. The bothrial grooves extend the entire length of the organ; they are usually, but not always, wider near the anterior end and are often convergent near the middle of their length. Gland cells, similar to those in Species No. 2, were observed in sections of the scolex.

There is no neck region; the scolex is followed immediately by the first proglottid, but does not overlap it. In this species the primary segments subdivide, so that new segments are intercalated in the strobila. In young specimens and especially in the anterior region of older ones, the division of the proglottids is often incomplete externally, while in older ones the genital rudiments are often separated into anterior and posterior portions before there is any external evidence of division of the proglottid. It is, therefore, impossible to count the proglottids with precision. There are always as many as can be recognized and probably additional ones, not yet distinguishable. In regions where division is occurring, the proglottids are alternately short and long; the shorter one being the anterior and the longer one the posterior portion of a primary segment. Even in strobilas with 500 or more proglottids, there were some, particularly near the ends of the chain, that were dividing. The proglottids are craspedote, the lateral margins serrate.

The longest worms measure about 180 mm in length and contain about 600 proglottids. A young complete worm, i.e., with terminal proglottid, 75 mm long, has 194 recognizable proglottids and no visible genital rudiments. Another specimen, 97 mm long has 226 proglottids, the last 5 of which are sterile and the preceding 38 have single genital rudiments (Fig. 15). Another complete worm, 132 mm long, has 470 proglottids; the genital rudiments are visible first in segment 230; they are double (i.e., one right and the other left) in this and the succeeding 155 segments; single in the next 82 segments; while the three terminal segments are sterile. Other specimens of similar size may have from 2 to 5 series of proglottids with doubled genital rudiments separated by series of proglottids with single or partially divided (Y or V-shaped) genital rudiments. Usually the segments at the posterior end of a strobila have single genital rudiments, although in a few the last fertile segments have partially or completely doubled genital rudiments. All of these specimens are juvenile; the testes and vitellaria, which appear first as scattered, discrete cell clusters, are not yet recognizable. The smallest complete, gravid specimen is much contracted; it measures 148 mm long and has 532 recognizable proglottids. The genital rudiments, which are double throughout, are first visible in segment 136; the testes at segment 224; the first eggs are in segment 300. In older specimens the first genital rudiments are nearer the scolex; in one worm in which the first genital rudiments appear in segment 106, the testes may be recognized in segment 200, but the first eggs are in segment 318.

The total length of one of these cestodes is of little importance, since a specimen can apparently contract to less than one-half its length when extended. As the strobila is contracted, the proglottids become shorter and wider. Two gravid and characteristic segments from the same strobila measure 3.44 by 0.57 mm and 3.12 by 0.78 mm. The following measurements in mm, of two specimens, one well extended and the other somewhat contracted, give the size of the proglottids:

	Specimen A		*Specimen B*	
Segment	*Length*	*Width*	*Length*	*Width*
1	0.14	0.78	0.1	0.82
50	0.52	1.20	0.16	1.51
100	0.58	1.56	0.20	2.40
200	0.58	2.20	0.26	2.70
300	0.65	2.34	0.32	4.0
400	0.71	2.8	0.33	4.15
500	0.72	2.8	0.48	3.91

In specimen A, the first genital rudiments are visible in segment 108 and the first eggs are in segment 290; in specimen B the genital rudiments are visible first in segment 148 and the first

eggs are in segment 332. In both worms, all gravid proglottids have doubled reproductive systems.

In a cross section of a gravid proglottid (Fig. 16), 2.65 mm wide and 0.69 mm thick, the cuticula is 0.006–0.01 mm thick; below the basement membrane there is a very thin sheet of circular muscle fibers; a single row of longitudinal fibers; then a cortical cellular zone, 0.14–0.195 mm in thickness, the outer portion of which contains scattered longitudinal fibers and the inner portion the vitelline follicles; then a loose parenchymatous layer about 0.067 mm thick in which there are longitudinal fibers; then a narrow band of transverse muscles, 0.01–0.015 mm thick; then the medullary zone, 0.17–0.195 mm thick, in which the testes, ovary and genital ducts are situated. There are very few dorsoventral fibers.

The excretory system consists of cortical and medullary longitudinal canals which extend throughout the strobila. The cortical ducts are more conspicuous, 10–14 on each side of the body in the vitelline layer and smaller ones more peripherally, but the number and arrangement are so variable that no definite pattern could be established. The principal ducts may converge and join as described for the previous species and connecting tubules between them form a network. There are two small medullary longitudinal ducts, located one on either side, mediad of the nerves. They have an undulatory course and receive segmental branches; whether these branches form segmental connections could not be determined, but it is probable that they do. The direction of flow in the excretory system is uncertain, but probably it is posteriad in the medullary ducts. In the terminal proglottid, the excretory channels are not constant or uniform. The two medullary ducts may converge and open into an excretory sinus which in turn opens to the exterior; or they may branch to form a plexus with which the cortical ducts communicate. The plexus may have two or more connections with the excretory sinus.

The genital rudiments (Figs. 13–14) appear first as short, deeply-staining rods of cells, located near the center of the proglottid. However, the genital rudiment may not be median and single; often it is bifid, usually at the anterior end, presenting a Y- or V-shaped appearance; while in more mature proglottids the rudiment is ordinarily separated into left and right portions, each of which gives rise to an independent set of reproductive organs. In gravid strobilas, a proglottid with a single set of reproductive organs is rare; occasionally there is a proglottid with a single ovary and two genital pores, undoubtedly the result of incomplete separation of the genital rudiments, but generally each gravid proglottid contains two complete sets of reproductive organs. The vitellaria and testes form continuous sheets of follicles in successive proglottids, so duplication of the central genital rudiments does not affect the arrangement of these structures; only their ducts are involved, the testes and vitellaria of one side discharge their products into the ducts of that side.

Examination of young worms discloses a peculiar developmental pattern in this species. The subdivision of proglottids and the duplication of genital rudiments afford a four-fold multiplication of the reproductive organs. The unfolding of the pattern apparently is correlated with age; single structures dividing in juvenile specimens, with complete duplication at maturity.

In those proglottids which contain two sets of reproductive organs there are two genital pores in each proglottid; in those with a single set there is only one genital pore; while in occasional proglottids there are two genital pores with the ducts from the two pores fusing to form single ducts. In such proglottids, the uterus and vas deferens are single in their initial portions and bifurcate with the branches leading to the right and left pores, respectively; conversely, there are two vaginae which fuse to produce a single duct that leads to the single seminal vesicle. The genital pores are oval, longer in the anteroposterior axis, located one-fifth to one-third of the length of the proglottid from the anterior end. The pores of the same segment are separated by a distance one-fifth to one-fourth the width of the proglottid. In a segment 2.68 mm wide, they are 0.6 mm apart. Each pore opens from a small, shallow, common genital atrium. The cirrus sac opens into the anterior, the vagina into the central, and the uterus into the posterior portion of the atrium; a condition similar to that described in Species No. 1.

The testes measure 0.04–0.08 mm in diameter and laterally occupy most of the medullary zone; medially they tend to lie in the dorsal portion of the zone; they are arranged in one, two, and sometimes three irregularly alternating layers. There are 20 to 40 follicles from one side of the proglottid to the other and 10–15 from the anterior to the posterior end. In young proglottids there are 5 to 10 between the cirrus sacs and other follicles extend posteriad on the dorsal side almost to the ovaries, but in fully gravid proglottids the testes of the two sides are continuous only on the anterior, dorsal side of the proglottid; otherwise they are absent in the median area which is filled with uterine coils. Vasa efferentia from the follicles converge toward the posterior end of the proglottid and on each side, just anterior to the ovary, they unite to form a long, much coiled vas deferens, filled with spermatozoa, which passes anteriad, dorsal to the uterine coils. At its anterior end the vas deferens constricts to a narrow duct which opens into the seminal vesicle, a muscular walled sac, 0.08–0.1 mm in length and 0.05–0.08 mm in diameter, which com-

municates with the duct that traverses the cirrus sac. The cirrus sac measures 0.2–0.25 mm dorsoventrally and 0.17–0.2 mm in diameter. The muscular wall of the cirrus sac is weak and continuous with that of the seminal vesicle, although the vesicle is not in the cirrus sac. In the dorsal portion of the cirrus sac the duct is coiled, and a straight tube, 0.2–0.24 mm long and 0.017–0.02 mm wide, lined with cuticula, passes ventrad and opens into the anterior side of the genital atrium.

The vitellaria are numerous, forming a continuous layer of follicles which is reduced or interrupted dorsally and ventrally only in the fields of the ovaries and uteri. Ducts from the vitellaria converge near the posterior end of the proglottid and, on each side, unite to form a common duct which passes ventrally and enlarges to form a vitelline receptacle which discharges into the oviduct of that side.

The vaginal opening into each genital atrium is just posterior to the opening of the cirrus sac. The vagina passes dorsally along the posterior face of the cirrus sac for a short distance, then expands and runs posteriad in a sinuous course below the uterine coils almost to the level of the ovary. It passes dorsal to the ovary in a Z-shaped fashion and the terminal loop is expanded to form a seminal receptacle. A narrow duct leads from the seminal receptacle to the oviduct. Each ovary consists of two wing-like lobes, connected by a narrower bridge. It lies near the posterior, ventral end of the proglottid, measures 0.3–0.5 mm in width, and the two ovaries of a proglottid almost touch each other. An oviduct arises from the median, posterior margin of each ovary; there is a conspicuous oöcapt; the oviduct coils posteriad, receives the duct from the seminal receptacle, turns laterad or mediad to receive the common vitelline duct, then posteriad and dorsad where it enters Mehlis' gland and enlarges to form the oötype. The initial portion of the uterus coils through Mehlis' gland, forms two or three transverse loops dorsal to the ovary and continues anteriad in transverse loops, 4–7 on each side, to the level of the genital pore where a long, narrow, straight duct passes ventrad and opens into the posterior side of the genital atrium. The course of the uterus can be traced only in young proglottids, before it becomes filled with eggs. The terminal loop in gravid proglottids is much expanded. Mature eggs measure 0.068–0.078 mm by 0.04–0.048 mm. In whole mounts and in sections, they are usually flattened on one side.

Species No. 4

Material of this species, from *Eumetopias jubata,* consists of many complete worms, both juvenile and gravid, and portions of strobilas. In general morphology they are very similar to the diplogonadial specimens from *C. ursinus*. They are certainly congeneric and, if the observed differences can be attributed to development in different host species, possibly specifically identical with those specimens. However, the worms from *E. jubata* are much larger and longer, more heavily muscled, have a distinctly different shaped scolex, more massively developed vitellaria, and fewer longitudinal excretory channels in the cortical zone. Consequently, in the absence of information concerning their life cycles, they are regarded as representatives of a distinct, but closely related, species.

The scolex (Fig. 18) is subglobular to quadrangular, with the walls of the bothria curved to produce a petal-like appearance. The scolex is typically thicker and wider anteriorly, tapering toward the posterior end. It measures 1.1–1.86 mm in length, 1.2–1.5 mm in greatest width and 1.7–2.1 mm in thickness. The bothrial grooves extend the entire length of the organ and are usually wider anteriorly. Many gland cells were observed. They are not apparent in whole mounts but in certain sections they are as numerous and as conspicuous as the gland cells of *Glandicephalus antarcticus* figured by Fuhrmann (1921).

There is no neck region; the scolex is followed immediately but the first proglottid, which is overlapped by it. The primary segments subdivide, as described in the previous species; subdivision is more active in young complete worms, and in the anterior one-fourth of older ones. The anterior portion of the strobila gradually widens to maximum breadth. The proglottids are craspedote, not markedly serrate laterally, relatively very short, and in contracted specimens the powerful longitudinal muscles produce a wrinkled appearance of the individual segments (Figs. 19–20). The posterior ends of the proglottids are thickened because of the velar overlapping of the succeeding segments.

In a complete specimen, 130 mm long, there are 516 recognizable proglottids. The genital rudiments are visible first at segment 162 and are double. The first eggs are in segment 246

and all succeeding ones except the last three contain eggs. The reproductive organs are double in all segments to 482, are single in the following ones except the last, which is sterile. In another complete worm, 178 mm long, there are 488 proglottids; the genital rudiments begin at segment 138 and the first eggs are in segment 166. The reproductive organs are double in all segments from 138 to 472; single in 473–487 with one sterile segment in this region; the last segment is sterile. All segments, except the sterile ones, from 166 to the end of the strobila, contain eggs.

An older, incomplete worm, i.e., without the terminal proglottid, 347 mm long, has 892 proglottids. The first genital rudiments are visible in segment 110; the first eggs are in segment 342, and all succeeding segments are gravid. The reproductive organs are double throughout. In another incomplete specimen, 352 mm long, there are 990 proglottids. The genital rudiments are recognizable first in segment 147; the first eggs in segment 282 and all succeeding segments are gravid, with doubled reproductive organs.

In representative specimens, the proglottids have the following measurements in mm:

Segment	*Length*	*Width*
1	0.18–0.28	0.72–0.97
50	0.22–0.3	0.1 –3.0
100	0.22–0.3	2.8 –3.4
200	0.22–0.3	3.4 –4.36
500	0.35–0.48	4.78–5.65
800	0.35–0.48	5.32–7.12

The cuticula is 0.014–0.024 mm in thickness. In a cross section (Fig. 21), 6.72 mm wide and 1.21 mm thick, below the cuticula there is a basement membrane, a very thin layer of circular fibers, a row of longitudinal fibers, and the cortical zone may be resolved into three layers of about equal thickness, a cellular layer, a vitelline layer, and a parenchymatous layer with longitudinal muscles; each layer about 0.11–0.14 mm in thickness. The transverse muscle sheet is very thin; 0.012–0.02 mm in thickness. The medullary zone is thicker in the fields of the ovaries and uteri where it measures 0.45–0.55 mm. Laterally the medullary zone becomes thinner, and may be only 0.12–0.16 mm in thickness.

The excretory system is difficult to trace. The longitudinal ducts of the cortical area, so conspicuous in the two previously described species, are visible occasionally in sections, but no definite pattern could be established. There are two very small ducts which run longitudinally in the medullary area; they are about 0.005 mm in diameter, situated one on each side, mediad of the longitudinal nerves. They are connected segmentally by tranverse ducts, 0.002–0.003 mm in diameter, that arise at the ovarian level and pass posterior to the ovary.

There are two sets of reproductive organs in mature and gravid proglottids; each proglottid has two genital pores, transverse oval or slit like openings, situated in transverse furrows, near the level of the posterior edge of the overlapping velum of the preceding segment. In contracted specimens, the genital pores are covered by the velum of the preceding proglottid. The pores are separated by about one-fourth of the width of the proglottid; in a cross section 6.6 mm wide, they are 1.65 mm apart. On each side, the cirrus sac opens into the anterior portion of a shallow genital atrium, the uterus into the posterior portion, with the vaginal opening between them.

The cirrus sac is usually compressed slightly in the antero-posterior direction; it is 0.24–0.3 mm in diameter and 0.3–0.4 mm long. The terminal portion of the duct is straight, 0.02–0.03 mm in diameter, and extends about one-half of the length of the cirrus sac. It is lined with cuticula and is continuous with a coiled duct, lined with epithelium, which traverses the dorsal one-half of the cirrus sac and communicates with the seminal vesicle which lies dorsal to the cirrus sac and measures 0.12–0.15 mm in diameter. The cirrus sac and seminal vesicle extend dorsoventrally across the medullary zone. From the seminal vesicle a short, thick-walled duct communicates with a thin-walled vas deferens which passes posteriad in loops and coils, dorsal to the uterine loops, almost to the level of the ovary where it breaks up into the vasa efferentia which lead to the testes. The testes are not delimited segmentally but form a continuous layer of follicles from one proglottid to the next. They tend to alternate and may be disposed in a single, double, or even three-layered arrangement. Since they are capable of changing position in the loose parenchyma, the arrangement of the testes obviously is influenced by contraction of the muscles of the proglottid. The follicles are oval to ovate, longer in the transverse axis, 0.07–0.14 mm in diameter; from anterior to posterior limits of a proglottid there are 10–16 follicles; 12–18 on each side lateral to the uterine coils, and in the anterior end of the proglottid 5–9 between the genital pores.

On each side, the vagina extends dorsad along the posterior face of the cirrus sac and turns posteriad in a sinuous course, below the uterine loops, almost to the level of the ovary;

here it turns dorsad and passes in a compressed Z-shaped manner above the ovary; the terminal loop is expanded to form a conspicuous seminal receptacle from which a small, short duct leads to the oviduct. In its anterior portion, the vagina is a narrow tube, 0.025–0.035 mm in diameter.

The vitellaria are very numerous, massively developed, and occupy the middle portion of the cortical zone (Fig. 21) except for the fields dorsal and ventral to the uterine coils. They extend from one proglottid to the next without segmental limitation. On each side, ducts from the vitellaria converge at the ovarian level and form a large receptacle, which may lie either mediad or laterad of the origin of the oviduct, and a short duct discharges into the oviduct.

The ovaries are located near the posterior, ventral border of the proglottid. Each ovary measures 0.85–1.3 mm in lateral extension. Medially the two ovaries may touch each other and laterally they extend beyond the limits of the uterine coils. The oviduct arises near the median posterior margin of each ovary and there is a distinct oöcapt. The oviduct passes posteriad, coils dorsad and either laterad or mediad, receives the duct from the seminal receptacle, turns to receive another from the vitelline receptacle, and coils posteriad and dorsad into Mehlis' gland. The 2 or 3 uterine loops above the ovary contain thin-shelled eggs, and anteriorly there are 5 to 8 loops on each side which contain thick-shelled eggs. The coils are so close together and superimposed that they can be traced only in young proglottids, before they become filled with eggs. The eggs are operculate, have thick shells, and measure 0.066–0.076 mm by 0.044–0.05 mm.

DISCUSSION

In view of the many reports made during the past century on cestodes of seals and sea-lions, it is probable that the species represented in the present material have been seen and studied by earlier observers, but as noted earlier, their descriptions are so incomplete and inadequate that, from their accounts, it is often impossible to recognize the species with which they were concerned. Moreover, descriptions of incorrectly determined specimens have added perplexity and dissension to the taxonomic problem. Consequently, allocation of the present specimens to previously described species is difficult if not impossible. The specimens from *Phoca barbata* were assigned earlier (Stunkard and Schoenborn, 1936) to *D. lanceolatum* (Krabbe, 1865) and that determination is probably correct. There are clearly two distinct species in the material from the fur-seals and a probable third species is represented in the specimens from the sea-lions. But until generic concepts are clearly formulated, it is impractical to attempt even generic determination.

In a repartition of the genus *Diphyllobothrium,* Mueller (1937) raised the subgenus *Spirometra* Faust, Campbell and Kellogg, 1929 to generic rank. He urged the reexamination of Cobbold's specimens of *D. stemmacephalum* in order to determine whether or not they are congeneric with *D. latum.* Until this question is definitely answered, the status of *Dibothriocephalus* Lühe, 1899, remains *sub judice.* Mueller suggested the recognition in *Diphyllobothrium* of three genera, based on the form of the scolex, presence or absence of a neck, structure of the copulatory organs, number and location of genital pores, shape of eggs, and type of intermediate host. *Spirometra* has a scolex and neck like *D. latum,* but it has separate male, vaginal and uterine pores, the seminal vesicle is included in the cirrus sac, the eggs are pointed, and the first intermediate host is a species of *Cyclops;* whereas *D. latum* has only two genital pores, the male and vaginal ducts discharging into a common atrium, the seminal vesicle is outside the cirrus sac, the eggs are oval, and species of *Diaptomus* serve as first intermediate host. A third suggested but unnamed genus, to which *D. lanceolatum* was referred, has no neck, the scolex is large and cordiform, each proglottid has a single genital atrium into which the male, vaginal and uterine ducts open, there is a separated seminal vesicle, and oval eggs. The characters listed by Mueller to separate three generic groups, although they appeared appropriate and

reasonable at the time, are rendered doubtful by the findings reported in the present paper. Our species No. 2 from *Callorhinus ursinus* has a cordiform scolex and a separate seminal vesicle like his genus 3, but has a short neck, and three genital pores like *Spirometra.*

The report by Mueller (1937) that scolices of specimens, similar to if not identical with *D. lanceolatum,* contain glands of the type figured by Fuhrmann for *D. antarcticum,* and our finding of such glands in the worms from fur-seals and sea-lions, raises a question concerning the validity of the genus *Glandicephalus* Fuhrmann, 1921 and possibly also of *Adenocephalus* Nybelin, 1931.

The problem of the seminal vesicle, whether or not it is included in the cirrus sac, is clarified at least in part, by the observation of Fuhrmann (1931) that the so-called seminal vesicle has a strong, muscular wall, continuous with but set off from that of the cirrus sac, and that this structure is not a seminal vesicle in the usually accepted sense, but an accessory copulatory organ, similar to that of the tetrarhynchids, for which the term "Propulsionsblase" was adopted. Fuhrmann's suggestion, that the "Propulsionsblase" may represent a secondarily separated portion of the cirrus sac, may explain the different conditions found in the diphyllobothrid cestodes.

Life history data are of primary importance for taxonomic considerations, but information of this nature is scanty and entirely lacking for the pseudophyllidean cestodes of the Pinnipedia. The type of crustacean utilized as first intermediate host is quite unknown and the course of development can only be estimated from specimens of different sizes and degrees of maturity. Whether or not a single species of these tapeworms can develop in more than one species of host and whether such development results in morphological differences, can be determined only by carefully conducted experimental studies. It is probably true that the diplogonadic condition has been derived from a monogonadic one; certain monogonadic species may show occasional diplogonadism (see Vergeer, 1935) and in diplogonadic species the juvenile and terminal proglottids may have single sets of reproductive organs; yet the secondary division of proglottids and the development of doubled reproductive systems in mature proglottids probably results from genetic changes that are specific if not generic.

The recent taxonomic revision of the cestodes from seals and sea-lions by Wardle et al does not commend itself as either sound or adequate. In their material they recognized four types: (1) a diplogonadic form from *Phoca groenlandica* and *Erignathus barbatus* of Labrador waters that resembled the description of *Diplogonoporus fasciatus* (Krabbe, 1865); (2) a mongonadic form from seals of Labrador waters that agreed with descriptions of *Diphyllobothrium lanceolatum* (Krabbe, 1865); (3) a diplogonadic form from *Callorhinus ursinus,* St. Paul's Island, Alaska; and (4) a second form, apparently monogonadic but not stated definitely, from *Callorhinus ursinus,* St. Paul's Island, Alaska. The descriptions are brief and incomplete in many respects. All the specimens were regarded as congeneric and for them the new genus, *Cordicephalus,* was erected. Types 1 and 2 were regarded as co-specific and identical with other diphyllobothrid species from seals in North Atlantic waters. All these forms were referred to *Cordicephalus phocarus* (Fabricius, 1780) with the following specific names reduced to synonymy: *phocarum* Fabricius, 1780; *phocae* Müller, 1780; *phocae foetidae* Creplin, 1825; *tetrapterus*

v. Siebold, 1848; *hians* Diesing, 1860; *cordatus* Leuckart, 1863; *elegans* Krabbe, 1865; *lanceolatus* Krabbe, 1865; *variabilis* Krabbe, 1865; *schistochilus* Germanos, 1895; *polycalceolus* Ariola, 1895; *römeri* Zschokke, 1903; *macrophallus* v. Linstow, 1905; *coniceps* v. Linstow, 1905; and *minus* Cholodkovsky, 1916.

Wardle et al reported that their type 3 was similar to *Krabbea grandis* Blanchard, 1894 (transferred to *Diphyllobothrium* by Lühe, 1899) and that their type 4 resembled *Diphyllobothrium arctocephalinum* Johnston, 1937 and *D. arctocephali* Drummond, 1937. All the worms, of both types 3 and 4, were regarded as members of a single species, *Cordicephalus arctocephalinus* (Johnston, 1937).

In the genus *Cordicephalus,* in addition to *C. phocarus* and *C. arctocephalinus,* Wardle et al recognized *C. tectus* (v. Linstow, 1892) with the following as synonyms: *scotium* Rennie and Reid, 1912; *perfoliatum* Railliet and Henry, 1912; and *lashleyi* Leiper and Atkinson, 1914. Also, provisionally, they included *C. quadratus* (v. Linstow, 1892) with the following species as identical: *resimum* Railliet and Henry, 1912; and *coatsi* Rennie and Reid, 1912. The genus *Cordicephalus,* Wardle et al, thus included species previously allocated to *Diplogonoporus* Loennberg, 1892; to *Krabbea* Blanchard, 1894; to *Dibothriocephalus* Lühe, 1899; and to *Diphyllobothrium* Cobbold as emended by Lühe, 1910. However, as noted by Stunkard (1947), *Taenia phocarum* Fabricius, 1780 (renamed *T. anthocephala* by Rudolphi, 1810) had been designated as type of the genus *Pyramicocephalus* by Monticelli (1890). The name *Cordicephalus* is therefore an absolute synonym of *Pyramicocephalus.*

Wardle et al stated, "The criteria upon which dibothriocephalid species are separated, namely—the shape of scolex, length and breadth of strobila, number and arrangement of testes as seen in a cross section, number of uterine loops, dimensions of intra-uterine eggs—are unreliable at best and especially so when applied to forms so muscular and contractile." Referring to the material which they studied, they stated, "The four types outlined above seem to lie within a single generic group, which like many pseudophyllidean generic groups, is highly variable in such details as scolex shape, relative thickness of cuticule, subcuticle, cortex, parenchymal muscle zone and medulla, number and arrangement of testes in cross sectional view, monogonadism and diplogonadism of genitalia, arrangement of uterine loops, according to the extent of contraction of the specimen and to its state of gonad development." The above statement concerning variation in diphyllobothrid cestodes is in part correct; in all these worms, muscular contractions induce changes in shape and in the relative form and position of parts while other changes, due to growth and to degrees of sexual maturity, are manifest by gradual alterations in the specimens. These variations are normal for any species, but they are not the features on which taxonomic determinations are made. Genetic differences, which distinguish between species, are manifest by real morphological differences, although it is often difficult to discriminate between true morphological differences and variations of a physiological nature. Examination of preserved specimens presents a further problem, since the condition in which the animal was killed may give a distorted idea of its normal appearance. However, the study of a large number of specimens, and at different degrees of maturity, will usually provide information on which sound judgments can be made.

In the present state of uncertainty concerning the morphology and ignorance of the life history of the cestodes from seals and sea-lions, the erection of a new genus, *Cordicephalus,* or the validation of *Pyramicocephalus* Monticelli, 1890, makes no contribution to knowledge of the genetic relationships or taxonomy of these species. Moreover, the species included in the genus constitute a heterogeneous collection of forms which lack real morphological unity. The differences among these species, as reported by many authors, are as pronounced as those accepted by Wardle et al to distinguish between other diphyllobothrid genera. Furthermore, it seems unwarranted, without supporting evidence, to reduce specific names to synonymy. It is almost certain that many of the described species are identical, but critical evidence has not yet been presented to determine which names are actually synonymous.

The treatment by Wardle et al of genera other than *Cordicephalus* also merits consideration. The genus *Diphyllobothrium* was restricted to two species, *D. stemmacephalum* Cobbold, 1858, the genotype, and *D. fuhrmanni* Hsü, 1935. These authors accepted the opinion of Hsü, that *D. stemmacephalum* of Yamaguti, 1935, is identical with *D. fuhrmanni;* but examination of the two descriptions indicates certain distinct differences. The description by Yamaguti gives no information concerning the total length of his specimens but the scolex is very different in form from that figured by Hsü; the proglottids are short and broad, about one-tenth the width, which was given as 20 mm, whereas the proglottid figured by Hsü is twice as long as broad and a maximum width of 8.5 mm was reported. Moreover, Yamaguti described two pairs of longitudinal excretory vessels in the medullary zone, whereas Hsü found only one pair in *D. fuhrmanni.* It seems obvious that the two forms are specifically distinct, and the worms described by Yamaguti from *Delphinus dussumieri* may be *D. stemmacephalum* or a closely related species. The genus *Diplogonoporus* Loennberg, 1892 was recognized, with only the type species, *D. balaenopterae,* which Wardle et al regarded as, "A doubtful form which may yet find its true position in *Cordicephalus.*" This statement further indicates that the proposed genus, *Cordicephalus,* is not clearly defined or delimited. If the type species of *Cordicephalus, C. phocarus* (Fabricius, 1780) and *D. balaenopterae* Loennberg, 1892 are congeneric, *Cordicephalus* is a synonym of *Diplogonoporus.*

Further study of the specimens from the fur-seal reported by Stiles and Hassall (1899) as *Bothriocephalus sp.* shows them to be specifically identical with one of the species collected from the same host by the U. S. Fish and Wildlife Service in 1935 and described in this paper as Species No. 2. Because of similarity in form of the scolex, von Linstow (1905) referred these specimens to his *Bothriocephalus macrophallus.* But *B. macrophallus* has a maximum length of 30 mm and width of 2.2 mm while the worms of Stiles and Hassall, from a different host and probably from a different region, have a maximum length of 120 mm and width of 6 mm. In *B. macrophallus* there is no neck and proglottids begin immediately behind the scolex. In the material of Stiles and Hassall there is a short unsegmented neck, the genital rudiments appear about 30 mm and the first eggs about 60 mm from the scolex. According to von Linstow, in *B. macrophallus* there are 50–60 longitudinal excretory canals in the cortical region and 20–24 in the medulla, the two sets connected by oblique anastomoses; a condition very different from that in Stiles and Hassall's specimens. In the figure of von Linstow, the medullary zone is thicker than the cortical zone, and the testes are represented in 2 or 3 layers. In his figure 14

the cirrus sac is more than one-half the width of the proglottid, although in the text the diameter is given as one-tenth the width and in figure 15 it is about one-seventh of the width of the proglottid. Von Linstow reported separate cirral, vaginal and uterine pores and in figure 14 they are represented as median and equidistant. In the specimens of Stiles and Hassall, the cirral and vaginal pores are near each other with the uterine pore laterad and some distance posterior to the others. It appears reasonably certain therefore, that the specimens of Stiles and Hassall are not specifically identical with *B. macrophallus*.

It is probable that the two species from *Callorhinus ursinus* described in this paper are identical with those described by Wardle et al as Types 3 and 4. The specimens are from the same host, the same locality, and in part at least from the same source, since Wardle et al reported receiving material from Victor B. Scheffer. Although the descriptions are not in complete agreement, our Species No. 2 is similar to their Type 4, which as they stated agrees closely with the descriptions of *D. arctocephalinus* Johnston, 1937 and *D. arctocephali* Drummond, 1937. The descriptions of Johnston and Drummond do not record any morphological differences and it is probable that their specimens are specifically identical. Whether the same parasite occurs in *Callorhinus ursinus* of the North Pacific and in species of *Arctocephalus* of the South Pacific is yet uncertain, but if it does, doubt is cast on the validity of other species which have been recognized, at least in part, by their occurrence in either Arctic or Antarctic seas. It is possible that detailed comparison of specimens from *Arctocephalus spp.* with material of our Species No. 2 may reveal specific differences. Our Species No. 3 is similar to Type 3 of Wardle et al. The species possibly should be referred to the genus *Diplogonoporus* and may be identical with *D. tetrapterus* (von Siebold, 1848), *D. variabilis* (Krabbe, 1865), or *D. fasciatus* (Krabbe, 1865). The subdivision of proglottids, reported for *D. variabilis,* is common in our material.

Our specimens from *Eumetopias jubata* (Species No. 4) resemble descriptions of *Diplogonoporus fasciatus* (Krabbe, 1865) more than any other species, but definite allocation can not be made until the description of that species is more complete.

It is certain that the specimens from *Callorhinus ursinus* comprise two distinct species and that the specimens from *Phoca barbata* described by Stunkard and Schoenborn (1936) represent a third species. Whether the specimens from *Eumetopias jubata* are identical with one of those from *C. ursinus* is yet uncertain but they probably represent a fourth species. The correct names for our species can not be determined until they have been compared with the several other previously named species, with one or more of which they may be identical. Unfortunately, previous descriptions are too incomplete to permit such comparisons at this time. The assignment of these species to genera can be made only when generic limitations are definitely determined and such a task will entail a taxonomic study of the entire group.

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EXPLANATION OF PLATES

ABBREVIATIONS

at	genital atrium	ov	ovary
cs	cirrus sac	sr	seminal receptacle
eg	egg	sv	seminal vesicle
ex	cortical excretory duct	ts	testis
gp	genital pore	up	uterine pore
lm	longitudinal muscle	ut	uterus
ln	lateral nerve	va	vagina
Mg	Mehlis' gland	vd	vas deferens
ml	medullary excretory duct	ve	velum
mp	male genital pore	vp	vaginal pore
od	oviduct	vr	vitelline receptacle
		vt	vitellaria

PLATE I

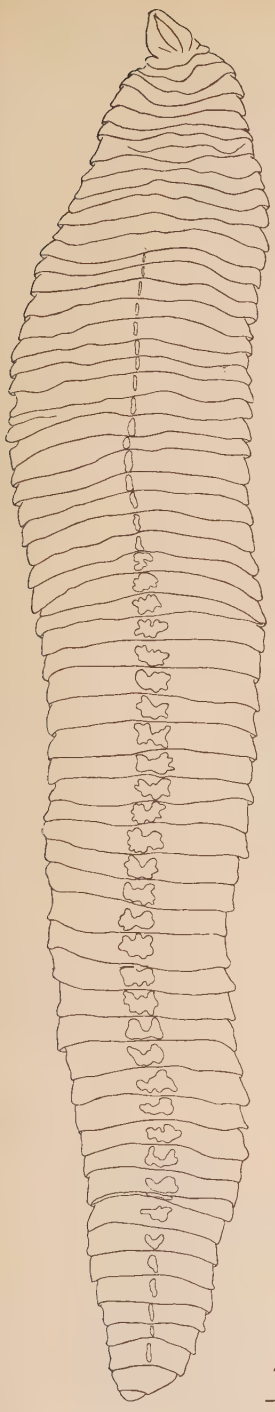
Species No. 1

FIG. 1. Entire specimen.
FIG. 2. Scolex and anterior proglottids.
FIG. 3. Sagittal section through three proglottids.
FIG. 4. Mature proglottid.

PLATE II

Species No. 2

FIG. 5. Scolex, slightly oblique view.
FIG. 6. Scolex, dorsal view.
FIG. 7. Juvenile proglottids, 118–120, showing genital rudiments.
FIG. 8. Cross section, at the level of the uterine pore.
FIG. 9. Mature proglottid and fenestrated strobila.
FIG. 10. Young proglottid, 140, few eggs in uterus.



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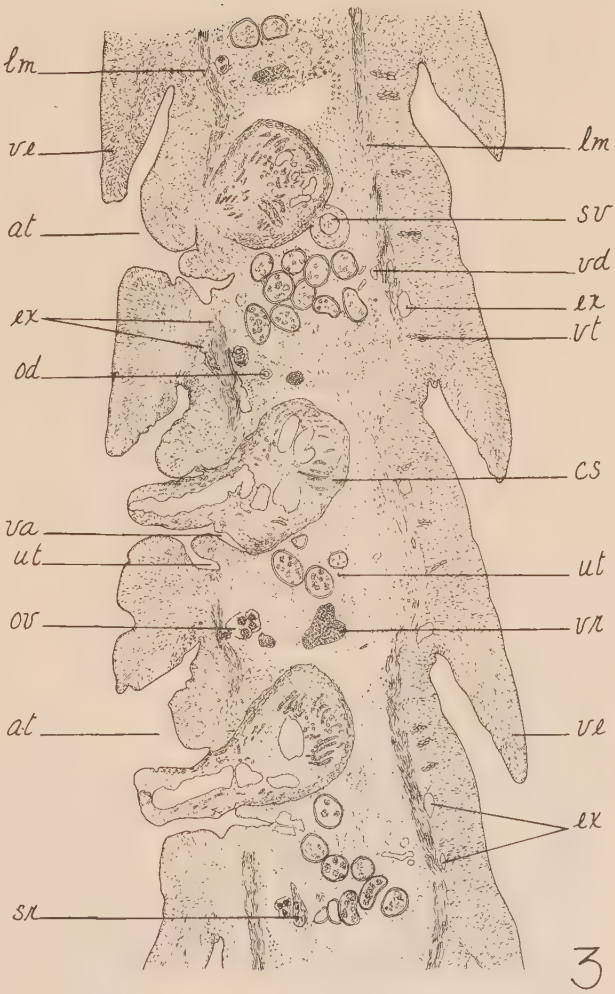
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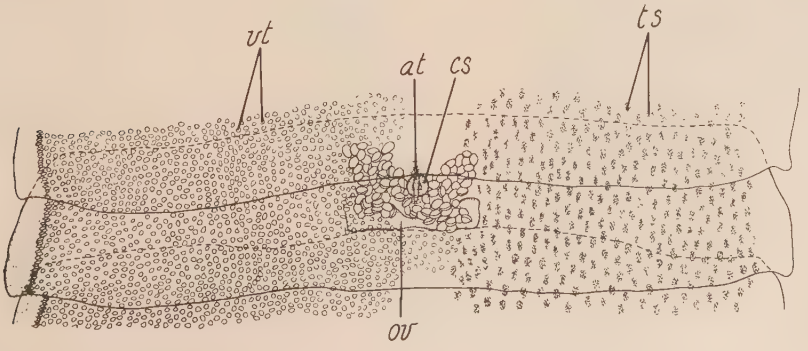
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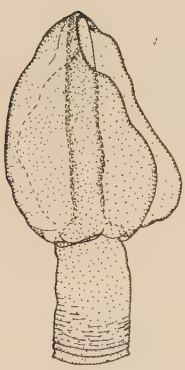
PLATE I



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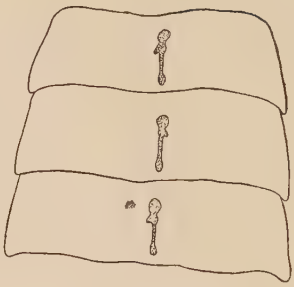

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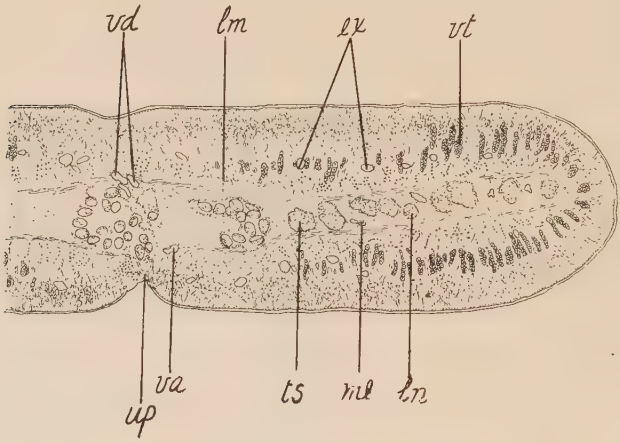

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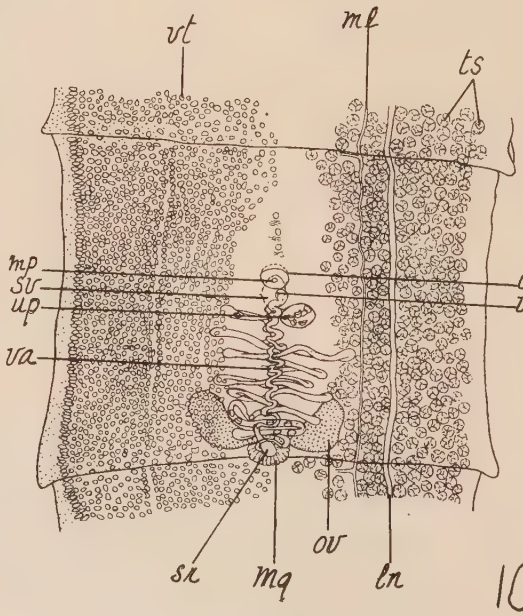

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PLATE II

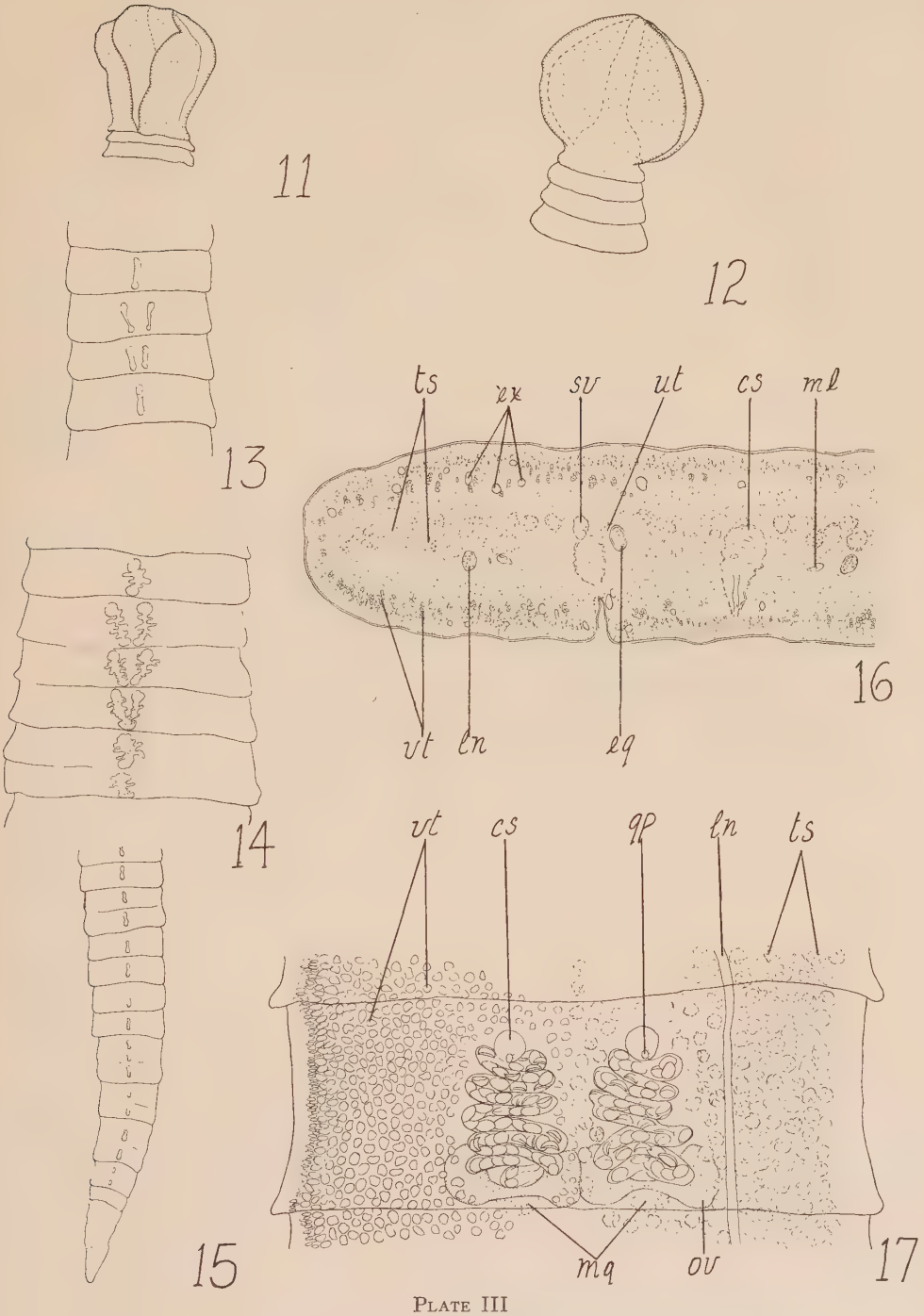
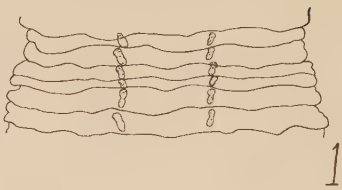


PLATE III

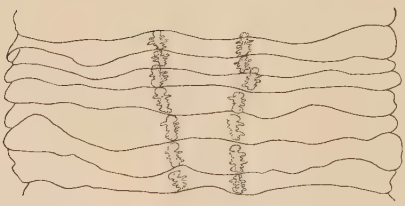
Species No. 3

FIG. 11. Scolex, dorsal view.
FIG. 12. Scolex, lateral view.
FIG. 13. Juvenile proglottids, showing genital rudiments.
FIG. 14. Juvenile proglottids, showing division of a proglottid and of the genital rudiments.
FIG. 15. Terminal proglottids, with dividing proglottids and genital rudiments.
FIG. 16. Cross section of a proglottid, at the level of the genital pores.
FIG. 17. Proglottid, not fully mature.

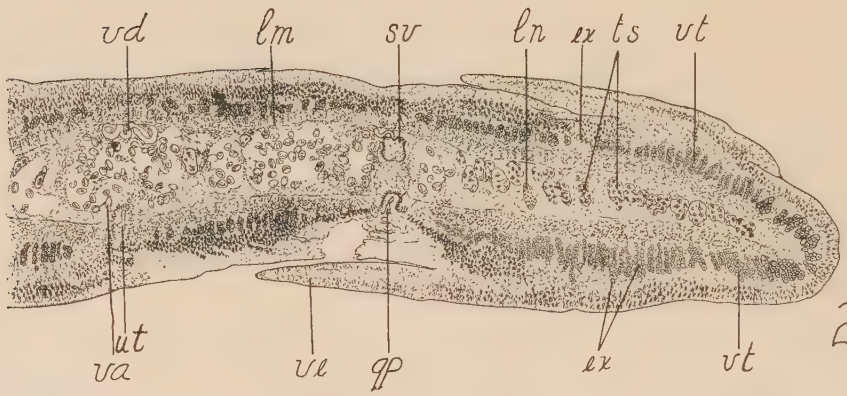
18



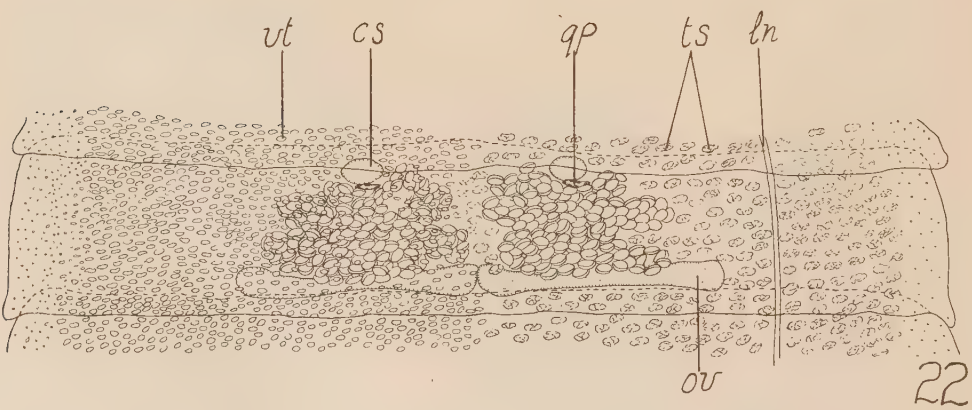
19



20



21



22

PLATE IV

Species No. 4

FIG. 18. Scolex and anterior proglottids.
FIG. 19. Juvenile proglottids, 160–165, showing genital rudiments.
FIG. 20. Juvenile proglottids, 297–303, showing form of segments and genital rudiments.
FIG. 21. Cross section of proglottid at the level of the genital pores.
FIG. 22. Mature proglottid, No. 452.

THE OCCURRENCE OF INTESTINAL PROTOZOA IN ADULTS IN SAN JUAN, PUERTO RICO

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Poindexter (1933) and Faust et al (1933) published the first extensive surveys of intestinal protozoa in Puerto Rico. While the first examined 564 specimens using direct smears and a culture method, the latter analyzed 1,003 stools, performing direct smears and flotation. Further survey data were not made available until Acosta Matienzo (1946) published the results of the examination of 1,780 fecal specimens, using direct smears only. Acosta Matienzo considered her percentages too low because of the method used and called attention to the need for a more adequate examination of the population of Puerto Rico. The surveys of Poindexter and Acosta Matienzo covered persons of all age groups from the area of San Juan and its suburbs, while Faust et al examined inhabitants chiefly from those parts of Puerto Rico where schistosomiasis is endemic. These areas do not include San Juan, except the nearby community of Rio Piedras. The report of Poindexter dealt only with *Entamoeba histolytica* and *E. coli.* In order to ascertain the present state of endemicity of protozoan intestinal infections in inhabitants of San Juan and its suburbs, a new survey was indicated.

TABLE 1.—*Intestinal protozoa in Puerto Rico*

Author	Poindexter	Faust et al	Acosta Matienzo	Present survey	
Year	1933	1933	1946	1948	
Population examined	San Juan, age?	Rural areas, all age groups	San Juan, all age groups	San Juan, adults without diarrhea	
Number of persons examined	564	1,003	1,780	600	
Findings	%	%	%	No.	%
E. histolytica	12.4	14.5	2.58	104	17.33
E. coli	46.0	34.2	18.2	206	34.33
E. nana	...	16.3	10.73	159	26.5
I. bütschlii	...	3.5	1.4	11	1.83
D. fragilis	...	...	....	2	0.33
Ch. mesnili	...	0.7	1.18	10	1.66
Trichomonas	...	...	1.35	14	2.33
Enteromonas	...	...	....	4	0.67
Embadomonas	...	...	....	2	0.33
G. lamblia	...	14.3	4.21	23	3.83
B. coli	...	0.2	0.22	1	0.17

The materials for the investigation were secured from the Presbyterian Hospital of San Juan. The patients of this hospital are mostly inhabitants of San Juan and its suburbs and represent all classes and age groups of the general population. One stool specimen was collected from each of the 600 adults examined. The time which elapsed between the evacuation and examination of the feces varied from a few minutes to one day. Dried-out material, incompletely examined stools, feces from children and from diarrheic patients were excluded from this study.

The examination of the stools consisted of microscopic search for protozoa in one saline suspension, one iodine-tinged smear (using D'Antoni's solution) and by zinc floatation (Faust). The preparation of permanent hematoxylin-stained slides ad-

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vocated and used by us in previous surveys (1945, 1946, 1947) could not be carried out because of the difficulties in securing adequately trained laboratory help.

Table 1 shows the results of this survey as compared with the findings of Poindexter, Faust et al, and Acosta Matienzo. *E. histolytica* was found more frequently (17.33 per cent) than by Poindexter (12.4 per cent). There is only a slight difference between the occurrence of *E. histolytica* in the out-of-town population examined by Faust et al 15 years ago (14.5 per cent) and the present rate of infection in adults living in the main city of the island. The smaller number of persons infested with *Giardia lamblia* (3.83 per cent) and *Iodamoeba bütschlii* (1.83 per cent) may be due to somewhat better sanitary conditions in San Juan and the exclusion of children from this survey. *Endolimax nana* is becoming more frequent in the Continental United States (Felsenfeld and Young, 1946). The larger number of inhabitants harboring this organism in San Juan (26.5 per cent) could be due to the frequent shifts of the population from and to Puerto Rico. As was expected, protozoa, with the exception of *G. lamblia,* were found more often in this survey than in that of Acosta Matienzo. The comparison of her statistics with ours shows the influence of the age groups (no children in the present survey) and the greater efficacy of Faust's floatation technic for the detection of *Entamoeba histolytica, E. coli* and *Endolimax nana.*

During the present survey a single species of protozoa was found in 207 (34.5 per cent), two species in 82 (13.66 per cent), three species in 51 (8.5 per cent) and four species in 3 (0.5 per cent) cases. Of the 600 persons examined, 343 (57.16 per cent) harbored protozoa. One has to expect that repeated stool examinations would have revealed a greater frequency of such infections.

The incidence of *E. histolytica* in the symptomless adults examined is definitely high and, if compared with the findings of Faust et al, has not receded during recent years. While frank amebiasis with the text-book clinical picture, i.e., type IV of Craig, is rarely encountered in San Juan and its suburbs, the frequent occurrence of *E. histolytica* shows that the danger of amebiasis persists in Puerto Rico.

SUMMARY

A single stool from each of 600 adult inhabitants of San Juan and its suburbs who did not show clinical signs of amebiasis was examined. Of the feces examined, 17.33 per cent harbored *E. histolytica.* The results are compared with statistics from previous surveys and with the findings of other protozoa in Puerto Rico.

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THE LABORATORY INFECTION OF *TRIATOMA NEOTOMAE* NEIVA WITH *TRYPANOSOMA CRUZI* CHAGAS AND SUBSEQUENT TRANSMISSION TO WHITE MICE

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Additions are constantly being made to the list of REDUVIIDAE from the United States which are either naturally infected, or can be infected under laboratory conditions with *Trypanosoma cruzi* Chagas.[2]

Kofoid and McCulloch (1916) were the first to report the discovery of a trypanosome in the digestive tract of a cone-nosed bug from the United States, *Triatoma protracta* Uhler, collected near San Diego, California. Subsequently Kofoid and Donat (1933) experimentally transmitted the infection from this species to the probable mammal reservoir, the wood rat (*Neotoma* sp.), while F. D. Wood (1934) experimentally infected many other species of mammals with *cruzi* from *T. protracta* Uhler collected from the same area.

Kofoid and Whitaker (1936) found *T. uhleri* Neiva collected near Tucson, Arizona, naturally infected with *cruzi,* and experimentally transmitted the infection by inoculation to the white-footed mouse (*Peromyscus* sp.). Wood and Wood (1938) collected specimens of *T. protracta* Uhler and *T. uhleri* Neiva in Arizona, New Mexico, Texas, and Utah, but none was found to be naturally infected. Specimens of both species, however, were experimentally infected in the laboratory by feeding on mice infected with *cruzi.*

Uninfected *T. sanguisuga ambigua* Neiva from Florida were experimentally infected by Packchanian (1940) by feeding on laboratory animals infected with *cruzi,* the trypanosomes from the insects establishing an infection when injected into uninfected mammals. Following this, Davis, McGregor, and de Shazo (1943) working in Texas found naturally infected *T. sanguisuga* (Le Conte) and *T. ambigua* Neiva (*T. sanguisuga ambigua* Neiva of Usinger (1944)) and succeeded in transmitting the infection to laboratory animals.

T. gerstaeckeri (Stal) and *T. heidemanni* Neiva (*T. lecticularius* Stal of Usinger (1944)) from various regions of Texas have also been found to harbor natural infections of *cruzi* which can be successfully transmitted to laboratory animals (Packchanian, 1939, 1940a). In addition, material from a naturally infected *T. heidemanni* Neiva(*T. lecticularius* Stal) has been used to establish a typical case of Chagas' disease in an adult male volunteer (Packchanian, 1943).

Wood (1941) reported for the first time natural infections with *cruzi* of *T. longipes* Barber from Arizona and *T. protracta woodi* Usinger from Texas, as well as a new locality in Texas for naturally infected *T. gerstaeckeri* (Stal). One specimen of *T. indictiva* (Neiva) from Texas was experimentally infected by him. Wood

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[2] *Trypanosoma cruzi* Chagas will be referred to as *cruzi* throughout this paper.

(1941a) has also collected *Paratriatoma hirsuta* Barber in both California and Arizona and succeeded in infecting three females with *cruzi* and transmitting this infection back to white mice.

Davis (1943) demonstrated that the fecal material of naturally infected *T. ger-*

FIG. 1. Adult of *Triatoma neotomae* Neiva.

staeckeri (Stal) from Texas, and of *T. protracta* (Uhler) from California, when dropped into the eye of a monkey (*Macacus mulatta*) gave rise to a unilateral, bipalpebral edema similar to the typical Romana sign described for South American cases in man.

Usinger (1944) lists *T. neotomae* Neiva as being reported in the United States only from the area around Brownsville, Texas. Although there are young nymphs in the type series reported as collected in Arizona, these are now recognized as being specimens of *T. rubida uhleri.*

According to Thurman (1944), specimens of *T. neotomae* have been taken by the Texas State Health Department in both Cameron and Willacy counties from nests of the wood rat (*Neotoma* sp.). He also refers to an unpublished thesis by Thelma de Shazo, who recorded finding specimens of *T. neotomae* Neiva in both the above mentioned counties naturally infected with trypanosomes of Chagas' disease, but no attempts to transmit the infection experimentally to a mammal have been reported in the literature.

MATERIALS AND METHODS

Dr. R. L. Usinger of the University of California checked the determination of specimens used in the present experiments and confirmed their identification as *Triatoma neotomae* Neiva. The adults (Fig. 1) run very well to this species in Usinger's (1944) key and agree with the description of *T. neotomae* in that paper except that Usinger states that *T. neotomae* is "naked except for fine pilosity on appendages," whereas these specimens show a short, golden-brownish, appressed pubescence on head, thorax, and abdomen, as well as the appendages.

The eggs of *Triatoma neotomae* Neiva (Fig. 2) have not been described or photographed previously. They are about 1.7 mm long by 1.1 mm in diameter. In Usinger's (1944) key to the eggs of TRIATOMINAE the eggs of *T. neotomae* would run to couplet 4 and fall with *T. dimidiata* (Latr.), *T. gerstaeckeri* (Stal), and *T. sanguisuga* (Lec.) in being two-thirds as long as wide and less than 2 mm long. The eggs are relatively smooth and shining, with hexagonal exochorial markings which are most easily seen after the young *Triatoma* has emerged from the egg.

A small colony of uninfected *Triatoma neotomae* was established at the Communicable Disease Center by Dr. Robert L. Usinger and Miss D. E. Harding in 1945. To maintain the colony, the insects usually were fed on laboratory animals at intervals of two weeks in summer, but at other seasons as much as six weeks intervened between feedings. Although nymphs would draw blood at shorter intervals, the adults seemed to prefer fasting for periods of at least two weeks. When sufficiently hungry, both nymphs and adults attack immediately in a bold fashion. As soon as the laboratory animal, such as a mouse, was detected, the *Triatoma* would raise its proboscis and tilt it slightly upward. Then with a swift, directed motion the insect would approach the animal and insert its beak. If a puncture was not made on the first attempt, the insect would withdraw slightly and then lunge forward again to penetrate the skin. Feeding usually continued for 20–30 minutes. When given the opportunity, uninfected nymphs and adults readily fed upon a human and would engorge completely.

Infection of T. neotomae *from Infected Mice.*—Two adults and three fifth-stage nymphs were fed on July 3, 1947, on white mice infected with a strain of *cruzi* which had been obtained from the Army Medical School. Seven days later an examination of the fecal discharges of all five insects showed an abundance of crithidial forms and occasional metacyclic stages. Films of the excreta of the triatomas were made by diluting with Locke's solution, air drying on slides and fixing in methyl alcohol. Ad-

ditional films were wet-fixed in formol as recommended by Elkeles (1945). All films were stained with Giemsa.

Attempted Infection of Mice by Biting Alone.—On July 20, these 5 bugs were again given the opportunity to feed, this time on uninfected mice. One of the nymphs was compelled to feed with its proboscis thrust upward at an angle through screening. In this case the mouse was placed above the insect and the only point of contact between the bug and the mouse was at the place where the mouth parts were inserted, thus preventing contamination of the mouse by any *Triatoma* fecal discharge. Engorgement took place in about 30 minutes. Upon removal of the nymph its abdomen was stroked gently which caused a large drop of dark brown

FIG. 2. Egg of *Triatoma neotomae* Neiva.

fluid to be expelled almost immediately. This excretion was diluted with a small quantity of Locke's solution and upon microscopic examination an enormous number of both crithidial and metacyclic forms were seen. The material was drawn into a syringe and injected intraperitoneally into a clean mouse.

Two other infected nymphs were fed similarly on another clean mouse, and after engorgement each was removed to a separate jar. Both soon defecated and examination of the excretion revealed an abundance of crithidial and metacyclic stages.

Blood examinations of all mice over a period of seven weeks showed only the mouse which had received the intraperitoneal injection of feces to harbor trypanosomes. These appeared on the seventh day after inoculation.

Approximately two months after the feeding had occurred, cultures of heart blood on N.N.N. medium were made from all the mice. In addition, bits of liver were

removed, using sterile technique, and similarly cultured. Positive cultures were obtained only from the animal which had been injected intraperitoneally with the excreta.

At this time, two months after feeding, the animals were sacrificed and autopsied carefully. The tissues all appeared normal on gross examination. Tissue sections of the heart, liver, skeletal muscles, and esophagus stained by a modification of the azure eosinate technique (Lillie 1945) did not reveal any evidence of infection in the mice on which the bugs had fed. Typical leishmania forms in cyst-like cavities, however, were encountered in various tissues of the infected mouse.

Infection of Mice from Contents of Triatoma *rectum.*—At the same time that the feeding experiments with the nymphs described previously were conducted, an infected adult *Triatoma* was given the opportunity to feed, but it refused to do so and was found dead a few hours later. The rectum was dissected out and its entire contents suspended in Locke's solution. Many crithidial and metacyclic forms were present. The suspension was injected intraperitoneally into two clean mice. Trypanosomes appeared in the blood of both mice seven days later. Seven weeks later positive cultures on N.N.N. medium were obtained from heart blood, and tissue sections of various organs showed typical leishmania forms.

The three types of excreta noted by Dias (1932) for *Triatoma megista* were also observed to be eliminated by *T. neotomae*. The fecal discharge was generally a viscous, dark brown material, while the yellowish liquid which was also deposited is interpreted by Dias as representing the malpighian secretion. Both of these types contained varying numbers of crithidia and metacyclic forms.

The third type, a clear, limpid, watery fluid was often formed soon after engorgement, and oozed out of the anal opening in droplets. On microscopic examination these invariably contained enormous numbers of crithidia and metacyclic stages. Rosettes composed of approximately a hundred organisms were also occasionally observed. It was noted that as the bugs engorged, both the swelling and increased weight of the abdomen would cause the posterior end of the *Triatoma* to drag on the surface. With the formation of these clear anal droplets following feeding, a trail of this exudate would generally be smeared on the surface as the bug began to crawl. Because of the huge number of trypanosomes present in such fluid, it appears that this may be very significant as a mechanism of infection of a mammal, especially when coupled with fecal deposition.

The trypanosomes which first appeared in the peripheral blood of the infected mice were both the very slender type, and the broader form. The former exhibited an extremely rapid, darting movement, quickly disappearing from the field, while the latter, although extremely active, tended to remain relatively confined within the field of view. During the first few days of the infection, there were about equal numbers of both types, but the broader form rapidly predominated.

Leishmania-like organisms were chiefly seen within the cyst-like cavities in the various tissues, but nests of fully mature trypanosomes were not infrequently found. Many cyst-like structures of leishmania stages were scattered throughout the heart musculature of the infected mice, as well as in the musculature of many of the organs, e.g., esophagus, trachea, and general skeletal muscles. The heart showed a typically diffuse myocarditis with an extensive interstitial edema and infiltration of lymphocytes, plasma cells, and histiocytes.

DISCUSSION AND SUMMARY

Experiments are reported in which attempts were made to determine whether *Triatoma neotomae* Neiva could transmit *Trypanosoma cruzi* Chagas by bite alone. All results were negative. Similar results were reported by Brumpt (1912) for *Triatoma megista,* and Denecke and von Haller (1939) for *Rhodnius prolixus* and *Triatoma pallidipennis.* However, inoculation of the excreta of infected bugs into mice always produced typical infections. Data are presented which indicate that nymphs and adults of *T. neotomae* are easily infected with *cruzi,* and that when the excreta of infected individuals are mechanically introduced into a mammal, infection will result.

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THE TREATMENT OF ASCARIASIS IN DOGS WITH 1–DIETHYL–CARBAMYL–4–METHYLPIPERAZINE HYDROCHLORIDE*

REDGINAL HEWITT, WYETH WALLACE, ELIZABETH WHITE, AND Y. SUBBAROW

It has been shown recently that 1-diethylcarbamyl-4-methylpiperazine hydrochloride produces marked effects against *Litomosoides carinii* in cotton rats (Hewitt, White, Wallace, Stewart, Kushner, and SubbaRow, 1947) and (Hewitt, Kushner, Stewart, White, Wallace, and SubbaRow, 1947), *Dirofilaria immitis* in dogs (Hewitt, Kushner, Stewart, White, Wallace, and SubbaRow, 1947), adult *Trichinella spiralis* in white rats (Oliver-Gonzalez and Hewitt, 1947), and *Wuchereria bancrofti* in man (Santiago-Stevenson, Oliver-Gonzalez, and Hewitt, 1947). The wide range of anti-helminthic activity exhibited by this compound has led to tests against other parasitic nematodes in animals and man. Data from fifty-one dogs infected with ascarids and treated with 1-diethylcarbamyl-4-methylpiperazine form the subject of this report.

MATERALS AND METHODS

Dogs from eight weeks to three or more years of age were used. No attempt was made to estimate quantitatively the number of ascarid eggs present before treatment. The compound was administered in capsules, in the various doses indicated in Table 1. Fasting prior to treatment or purgation following treatment were not

TABLE 1.—*Summary data from 51 dogs treated with 1-diethylcarbamyl-4-methylpiperazine hydrochloride for ascariasis*

Dose (mg. per kg.)	No. of dogs used	Total no. of ascarids passed	Ascarids found at autopsy (intestine)	Efficiency of treatment (per cent)
12.5 Oral 2 doses	6	52	7	88.2
25 Oral 1 dose	7	25	13	65.7
25 Oral 2 doses	7	62	None	100.0
30 Oral 1 dose	6	11	1	91.7
40 Oral 1 dose	7	56	18	75.7
50 Oral 1 dose	10	72	1	98.7
50 Oral 2 doses	4	33	None	100.0
50 I.P. 2 doses	4	9 (plus)	None	100.0

Total No. of dogs treated—51.
Total No. of ascarids passed—320 (plus).
Total No. of ascarids recovered from intestine (all dosages)—40.

practiced. Following dosage, the dogs were isolated in individual cages, and whenever possible all stool passages were examined for the presence of worms for at least two or three consecutive days. Examinations for eggs were made within two to thirteen days after treatment, and necropsies were performed within the same period. The entire intestine was examined for the presence of worms.

RESULTS OF TREATMENT

Ascarids were passed in the feces or vomitus within a few hours to four days after the capsules had been given. The most effective oral doses were 25 mg per kg given twice, or 50 mg per kg administered once (Table 1). When two doses were

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*From the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York.

administered, the first capsule was given in the morning and another at late afternoon or the first capsule was given in the afternoon and the second on the following morning. A single dose of 30 or 40 mg per kg was nearly as effective as 50 mg per kg, although one dog in the 40 mg per kg group (No. 370) retained twelve worms. It is possible that this animal regurgitated the capsule.

Treatment was 100 per cent effective in the group given 50 mg per kg intraperitoneally for two doses. A summary of the results obtained is given in Table 1.

TOXICITY

Approximately one-half of the dogs vomited after the first or second dose, but no further symptoms occurred which could be attributed to the drug. One dog which received 12.5 mg per kg for two doses orally (No. 909) died from an unknown cause on the third day. When vomiting occurred after treatment, worms were frequently expelled in the vomitus.

Previous studies with this compound in dogs infected with heartworm (Hewitt, Kushner, Stewart, White, Wallace, and SubbaRow, 1947) demonstrate that it is well tolerated for long periods of time without demonstrable toxicity of serious nature. Dogs treated with 25 mg per kg three times daily for from two weeks to two months, gained in weight, behaved normally, and showed few signs of discomfort with the exception of occasional and transient nausea and vomiting. Tissues from two dogs treated with this dosage for two months revealed nothing remarkable at autopsy. Further studies in various other laboratory animals (Harned, Cunningham, Halliday, Vessey, Yuda, Clark, Hine, Cosgrove, and SubbaRow, 1948) demonstrate that the drug is practically non-toxic in the therapeutic doses used, and that mice, rats and dogs will tolerate doses many times in excess of the amount which has been shown to be effective for removing ascarids from dogs.

REMARKS

1-diethylcarbamyl-4-methylpiperazine hydrochloride is relatively non-toxic, and the danger of using too large a dose in dogs is slight. The compound is excreted rapidly and is apparently not irritating to the intestinal mucosa. Of singular interest is the fact that the intraperitoneal method of administration is also effective in removing the worms.

Many of the dogs used for the present tests also harbored hookworms, whipworms, and tapeworms. No measurable effect has been produced against any of these other worms.

The possible value of this compound for treating ascariasis in human patients is not known as yet, although preliminary data from six patients indicate that it does produce an effect against *Ascaris lumbricoides* (Oliver-Gonzalez and Hewitt, In press).

SUMMARY

Data are given from fifty-one dogs infected with ascarids and treated with 1-diethylcarbamyl-4-methylpiperazine hydrochloride, Single oral doses of 50 mg per kg were 98.7 per cent effective, and two oral doses of 25 mg per kg within a 24-hour period were 100 per cent effective. Two intraperitoneal doses of 50 mg per kg were 100 per cent effective. The compound is relatively non-toxic to dogs. Fasting preceding treatment and purgation following treatment were not performed.

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HERMAPHRODITES IN A PUERTO RICAN STRAIN OF *SCHISTOSOMA MANSONI*[1]

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In the fall of 1946 some interesting sexual anomalies were observed in certain males of a lot of *Schistosoma mansoni* which were being stained and mounted whole for class use. A study of the worms in question revealed that they possessed ovaries in various degrees of development in addition to testes. These observations were later confirmed by an examination of serial sections. Of the 35 males studied, 13 exhibited this hermaphroditic condition. These worms were purchased from the South-Western Biological Supply Company which in turn had secured them from a collector in Santurce, Puerto Rico. Nothing is known concerning the host or the age of the worms.

Recently Vogel (1947) described, from whole mounts, secondary hermaphrodites of male *S. mansoni,* some of which had well-developed ovaries, and, in addition, oviducts, uteri, and vitellaria in various stages of completeness. However, he did not find vitelline ducts, oötype, Mehlis' gland or a female genital pore. He described the uteri as ending blindly, usually on the ventral side of the worm just beneath the covering of the body at the level of the anterior testes. He stated: "A female genital pore could never be made out with certainty, although in one specimen a short cord of tissue was seen connecting the conical end of the uterus with the ventral skin close to the male genital opening, probably representing a rudimentary female genital pore." He interpreted the absence of vitelline ducts as "a fact which points to the conclusion that production of ova is impossible in these hermaphroditic males." Since some of our males have more completely formed female reproductive systems than any described by Vogel and since our specimens are from Puerto Rico and at least a part of Vogel's are from West Africa (the source of all his specimens is not stated), it is thought worth while to report briefly on our findings at this time. A more detailed description will be given in a later paper.

The female organs in our hermaphroditic males vary considerably with respect to size, position and state of development, no specimen possessing a typically developed female reproductive system. In general our findings agree with Vogel's description and figures. The ovary of one specimen is situated at the level of the second and third testes, only a slight distance behind the seminal vesicle, while in other worms the ovaries are father posterior, near the junction of the intestinal ceca. Some males possess small ovoid ovaries, with accessory female sex organs partially or entirely absent. Others, in addition to rather well-developed ovaries with typical oöcytes, have oviducts, oötypes, Mehlis' glands, short uteri, main vitelline ducts (two worms) and vitellaria (at least one worm) in different stages of completeness. A female genital pore is visible in three of our specimens, in one of which no connection is evident between the rudimentary ovary and the oviduct. In the specimens with a

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readily visible genital pore the male and female ducts unite either at or very near the orifice, which may be considered to be a common genital pore. Four males have oötypes and Mehlis' glands; although in certain of these specimens these structures are not fully formed. In an additional specimen, which has no Mehlis' gland, a dilatation of the female genital duct appears to be a poorly formed oötype. Two males, one of which was fixed *in copula* with a normal female, possess especially well-developed female reproductive systems (Fig. 1). The specimen drawn has a complete set of female genital organs, although the vitellaria are not well formed, and the uterus, oviduct and main vitelline duct are not typical, in that the uterus is shorter and the last two ducts proportionately longer than in normal adult females. In this specimen the terminal part of the uterus connects with the ejaculatory duct very near the ventral surface of the worm.

Vogel found the oviducts of his hermaphrodites empty, except for one which contained two egg cells. The female genital ducts of our specimens are devoid of

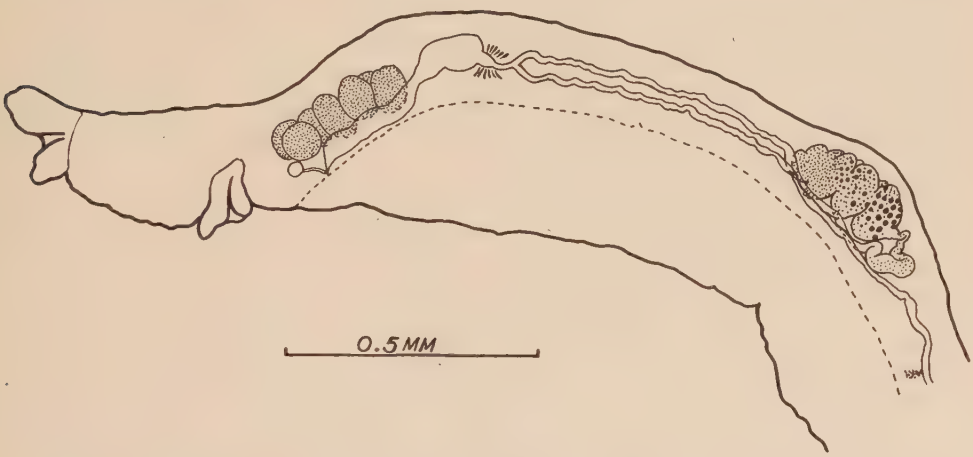

FIG. 1. Hermaphroditic male *Schistosoma mansoni*. Drawn with the aid of a camera lucida from whole mount and serial sections.

spermatozoa. Most of the oviducts, however, contain small amounts of granular material which appears pinkish after staining with eosin. Four contain, besides this granular material, some cells which are not typical oöcytes, but are for the most part small cells, degenerate in appearance. These cells may be oögonia or atypical oöcytes liberated prematurely from the ovary. Some oötypes and uteri contain minute eosinophilic globules and small cells which appear to be dead and disintegrating. These cells probably correspond to the accumulation of cellular material which Vogel found in the terminal portion of the uteri of his specimens. At least a few of the eosinophilic globules seem to be secretions of the Mehlis' gland. No eggs (i.e., eggs with shells) were found in the oötypes or uteri of our hermaphrodites, but it is of interest that there are three vitelline cells in the main vitelline duct of the male of fig. 1, and the uterus of another specimen contains an ill-defined mass which appears to consist of disintegrating or dead cells and unorganized shell material. However, the possibility that these males have formed and deposited well-formed eggs seems remote because of the slight development of the vitellaria.

Vogel found that the majority of his hermaphrodites had normally developed

testes and seminal vesicles filled with spermatozoa. But in some specimens the female organs had apparently developed at the expense of the male glands. An examination of our hermaphrodites revealed a few spermatozoa (possibly only spermatids) in the seminal vesicles of only two worms. The seminal vesicles of three, including the two just referred to, contain a small number of cells which are not spermatozoa or spermatids, but seem rather to be spermatogonia and spermatocytes, some of which are apparently disintegrating; while in others, chromosomes are visible, indicating some phase of nuclear division. A reduction in the number of testes is evident in four worms of our collection, two having only three testes each. In no specimen, however, is the suppression of the male organs as great as in Vogel's fig. 1, c.

Since the age of our specimens is unknown, we are not certain whether the absence of spermatozoa in the seminal vesicles of most, if not all, of them is primarily a result of immaturity of the worms, or of an inhibitory effect exerted by the female organs. The fact that Vogel found spermatozoa in the seminal vesicles of most of his hermaphrodites indicates that the testes can mature in the presence of the female organs. If the immaturity of the testes of our specimens is a result of immaturity of the worms, the already rather completely developed female organs in at least two males causes one to speculate whether viable ova would have been produced, had the worms lived longer.

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INVESTIGATIONS ON THE LIFE CYCLE OF *OÖCHORISTICA RATTI*, A CESTODE FROM RATS AND MICE*

R. C. Rendtorff

In a preliminary report on the life cycle of a cestode of the mouse the author (Rendtorff, 1939) stated that the tapeworm closely resembled members of the Anoplocephalan genus *Oöchoristica*. Further investigations and particularly the discovery that the rat can also serve as the definitive host led to the conclusion that this tapeworm is *Oöchoristica ratti* Yamaguti and Miyata, 1937. The specimens from which they described the species taken by Yamaguti and Miyata from the small intestine of *Rattus rattus rattus* and *R. r. alexandrinus* captured aboard Taiwan and Shanghai liners.

MATERIALS AND METHODS

Cestode material was originally obtained by trapping wild mice in the Natural History building, Urbana, Illinois. Tapeworms were removed from the definitive host by necropsy. Ripe proglottids were separated for infection experiments and the remainder of the worm was fixed in corrosive sublimate or a mixture of a low concentration of acetic acid in formalin and alcohol. The stains, acid carmine, alum cochineal, and a 50 percent mixture of Delafield's and Ehrlich's haematoxylin, were satisfactory.

Cultures of insects were reared free from rodent contamination in accordance with well-known methods. Cockroaches were obtained from egg capsules and reared in crystallizing dishes. Stock cultures of grain insects were kept in cloth battery jars containing a cereal medium described by Hydak (1936). These insects were transferred to Petri dishes for infection experiments. Most species of insects readily accepted a mixture of ground proglottids in dry Hydak's medium which was very slightly moistened. If this mixture was too moist, insects fed poorly and detrimental molds occurred. Large insects, especially meal worms, accepted the ripe proglottids from the end of a probe or between two flakes of rolled oats.

Stock cultures of Oribatid mites were kept in large stender dishes containing a small amount of soil from which they were collected. The mites were fed broken bits of meal worm larvae and vegetable matter. If excess decay or mold appeared in the cultures it was removed. Mites which were to be infected were removed to a clean stender dish, or placed within a chamber made by sealing a glass ring, measuring three-quarters of an inch in diameter by one half an inch tall, to the bottom of a Syracuse watch glass and then inserting a circle of blotting paper in the floor of the ring and covering with a cover slip. Mites were offered whole proglottids or feces containing them. Dishes containing the mites were placed within a desiccator containing some water to maintain humidity in the cultures.

For an examination of living developmental stages of the cestode, the body con-

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tents of the infected insects were dissected out onto a microscope slide, a little water or physiological saline solution was added and then covered with a cover slip. In order to prepare fixed specimens of these stages the insects were killed in boiling water, removed to A F A or Bouin's fixative, washed thoroughly in alcohol, dehydrated by passing through either a series of alcohols or in Dioxan, cleared and then placed in a five percent solution of celloidin in methyl benzoate. From this last mixture they were imbedded in a series of paraffins and then sectioned. Haidenhain's iron-alum haematoxylin and Delafield's haematoxylin gave satisfactory stains with the insect material.

Laboratory albino rats and mice were maintained free from insect contamination. These hosts were infected by placing them in a battery jar and offering them the infected living insects, or occasionally insects crushed and mixed with food.

RESULTS

The intermediate host—Direct infection experiments on mice and rats were first attempted. As these experiments proved negative in every case a survey was made to determine the probable intermediate host. Household insects and pests of stored grain and food products were considered to be the most likely hosts because of their

TABLE 1.—*Results of feeding eggs of* Oöchoristica ratti *to suspected intermediate hosts*

Specific name	Common name	Life stages examined*	Cysticercoids
Galumna sp.	Beetle mites, etc.	N A	Absent
Blatta orientalis	Oriental cockroach	N	Absent
Trogoderma versicolor	Museum beetle	L P A	Present
Attagenus piccus	Black carpet beetle	L	Present
Anthrenus verbasci	Buffalo carpet beetle	L	Present
Tribolium confusum	Confused flour beetle	L P A	Present
T. ferruginеum	Rust-red flour beetle	L P A	Present
Tenebroides mauritanicus	Cadelle	L P A	Present
Tenebrio molitor	Yellow meal worm	L A	Absent
T. obscurus	Dark meal worm	L	Absent
Undetermined sp.	Beetle	A	Present
Plodia interpunctella	Indian meal moth	L P A	Present

* L—larva, P—pupa, A—adult, N—nymph.

general availability to mice and actual presence where infected mice were found. Oribatid mites were considered as possible hosts in view of Stunkard's (1937 to 1941) findings that these mites serve as intermediate hosts for certain of the Anoplocephaline tapeworms.

The results obtained by experimentally feeding onchospheres to arthropods is shown in Table 1. It should be noted that the stages of the arthropods which were examined are not necessarily the stages at which they became infected. All species of insects which were capable of being infected were infected during some larval stage. Of these, attempts to infect first larval stages were made only with *Trogoderma versicolor,* and these were successful. In addition to these, attempts to infect adult stages of *Tribolium* were successful. Those species in which no cysticercoids are reported failed to become infected upon repeated trials.

The first attempts to infect insects were made on *Tenebrio molitor* and *T. obscurus* larvae. Repeated examinations of larvae fed gravid proglottids failed to show infection. However, examinations of the insects' feces one to two days after they ingested eggs revealed many hatched embryos. Apparently these embryos found the tissues of the intestine impenetrable. Of the other insects, *Blatta orientalis* nymphs were also unable to serve as intermediates.

Upon examination of Oribatid mites a few days after exposing them to gravid proglottids it was often found that the food ball within the ventriculus contained several hexacanth embryos. These, however, were either disintegrated as though torn apart during mastication, shriveled, or abnormal in other respects. Of 68 mites examined at various intervals up to one month after exposure to eggs, none was found infected. The author feels the technic employed in experimental infection of the mites was reliable for in one control experiment using *Moniezia expansa* a very early stage of a developing cysticercoid of that species was found after 11 days within the same species of mite used in experiments with *Oöchoristica ratti.* The author has identified the mites as belonging to the genus *Galumna* but possibly other mites were also present. Further investigations are necessary before the status of Oribatid mites as intermediate hosts for the members of the genus *Oöchoristica* can be definitely established.

Experimental infection showed members of the orders COLEOPTERA and LEPIDOPTERA capable of serving as intermediate hosts. Of the COLEOPTERA studied three species belong to the family DERMESTIDAE and three species to the family TENEBRIONIDAE. An undetermined beetle believed to belong to neither of these families was also infected. This beetle, a single specimen, was less than 2 mm. in length and was collected along with Oribatid mites from a soil sample.

The insects capable of transmitting the parasite have in common with the definitive host, habitat and food habits, that is, they live in and feed on cereal products. The insects are, therefore, readily available to rats and mice and are indeed relished by them. Gravid proglottids passed in the feces of these rodents may contaminate cereal products and being slightly moist are attractive to insects. It is logical then, that these insects serve as intermediate hosts in nature although they have not as yet been found naturally infected. It is very likely that the list of intermediate hosts will be extended upon further study.

Distribution of the tapeworm may occur through the activities of the insect or rodent hosts, or through transportation of either of these by man. The only previous published record of this tapeworm is that of specimens taken from rats aboard ocean vessels in Japan. In Urbana, Illinois, the parasite seems to have a limited distribution. Dr. Lyell J. Thomas first encountered it in 1921 in the Natural History building and states that for many years rodents of various kinds and feeds and grains have been kept in the animal room in the basement of the building. This is the same area from which the present writer collected his material. Mr. Cook, one of Dr. Thomas' students, also found this tapeworm in mice caught in the Natural History building but failed to find it in his survey of mice from adjacent and relatively distant buildings.

The cysticercoid.—The gravid proglottids contain eggs singly in a series of uterine capsules. Each onchosphere (Fig. 1) is contained within a thin outer shell 46–51 μ long by 35–38 μ wide and a thick embryonic shell 32–35 μ long by 27–30 μ wide. The embryo is 30–32 μ long 24–27 μ wide and hooks are 14–16 μ long (Fig. 2).

Upon being taken into the alimentary tract by a suitable insect the hexacanth embryo is freed from the outer shell and penetrates the wall of the ventriculus. This is evidenced by finding hexacanth embryos lining the surface of the ventriculus 48 hours after exposure to infection (Fig. 4). The hexacanth embryos lining the sur-

face of the mid-gut at this time differ little from the embryos within the eggs and measure 32–36 μ long by 29–30 μ wide. The appearance is hyaline throughout without much cellular differentiation. The occasional movement of the hooks is the only motion observed during this stage although numerous embryos are found in various stages of contraction at the surface and between the circular muscle bands of the ventriculus. In a single instance a hexacanth embryo, four days within the insect, was observed to have two elliptical areas approximately equal to the length of a hook (Fig. 3). These areas were lateral to and below the hooks and were denser and more granular in appearance than the surrounding substance. Subsequent to this observation eggs were examined after intra-vital staining with bromcresyl blue. The areas described are brought out distinctly by the stain but appear more rounded in the onchospheres within the eggs than in the hatched embryos. The granular nature of these areas and their comparatively large size is striking; their function is unknown.

After entrance into the haemocoele, within three or four days after ingestion by the insect, the embryo undergoes a period of growth (Fig. 5). The cells at first increase in size and number but remain undifferentiated. By the seventh day within the insect there can be seen a well-defined centrally located area composed of larger clearer cells (Fig. 6). This would seem to be the beginning of scolex formation. The cuticula, or body wall, is becoming thicker and evidence is found of fine irregular lines on the surface running as parallels about a sphere. Up to the beginning of scolex formation the cysticercoid is usually spherical but as it develops it tends to become bluntly ovoid. Forms showing the earliest stages of scolex formation measure 94 μ long by 87 μ wide. Scolex development continues at a comparatively rapid rate. In one experiment during a hot spell in the summer, cysticercoids with apparently fully developed scolices were found in an insect nine days after infection. The developing cysticercoids become more active until they are mature and even then they often contract and extend continuously when dissected from the insect. Measurements of the living, fully developed cysticercoids vary according to the state of contraction as well as with the actual size. At rest the body is bluntly ovoid, the scolex end being more rounded than the opposite end. The average measurements of six fully developed cysticercoids 60 days within the insect are as follows: total length, 180 μ; total width measured across the organism on a line through the center of the suckers, 141 μ; length of suckers, 56 μ; width of suckers, 42 μ; distance across the outermost suckers, 105 μ; body wall, 4 μ; calcareous bodies up to 9 μ.

The fully developed cysticercoid (Fig. 7) retains the embryonic hooks and these are found at the posterior region in less orderly arrangement than in the embryo but still roughly grouped in pairs. The scolex is invaginated and distinctly visible; less easily discernible is a minute invagination at the opposite pole. The latter appears to be a duct which drains to the exterior from a tiny reservoir or bladder into which the four main ducts, two dorsal and two ventral, of the excretory system flow from above. Originating from this reservoir the excretory ducts extend in two lateral pairs toward the scolex. They follow the general contour of the body wall until they have approached the scolex where they then bend back upon themselves and progress down along the invaginated scolex. These ducts terminate, as in the adult tapeworm, in a single excretory ring in the scolex. The excretory ducts are often convoluted, particularly in the region where they bend near the invagination

of the scolex. When the scolex is evaginated these ducts straighten out and follow a course identical to the excretory system in the mature tapeworm, indeed, fine cross commissures and anastomoses between main excretory ducts are discernible in the evaginated cysticercoid (Fig. 8).

Cysticercoids dissected from *Trogoderma* larvae were occasionally found to be completely enveloped within a very thin membrane which in one instance was twice the width and almost twice the length of the fully formed cysticercoid within it. Usually the envelopes appeared distended and filled with a clear fluid though occasionally they were shrivelled and collapsed about the cysticercoid. One such membrane irregularly enclosed three cysticercoids. In some cases a small cluster of cells with large nuclei were also within this membrane (Fig. 11). Upon examination with an oil immersion lens this envelope was, in one instance, found to contain two large cells along its inner surface, the cells being very granular, dense, and containing large nuclei (Fig. 10). Quite often in sectioned material a clear area would be present between the cysticercoid and host tissue and the indication of a thin membrane was present in some such cases; but the large cells were not found and hence the membrane could not be proven to exist. No evidence as to the function or origin of this membrane can as yet be offered. It is not consistently present and would seem to be most likely of host origin. The effect of this membrane upon the infectivity of the cysticercoids is not known; morphologically they appeared identical to infectious forms. There is no evidence to believe that any of the cysticercoids are affected by the normal host in such a manner as to produce mortality or render cysticercoids non-infectious. Likewise no mortality was observed among the insects that could be credited to infection by this parasite. Another interesting relationship which was not further investigated was found in a simultaneous infection of *Trogoderma* larvae with *Oöchoristica* and an undetermined species of GREGARINE. The GREGARINE, probably a SCHIZOGREGARINARIA, produced innumerable large spores which caused a very high mortality among the insects. Cysticercoids found in conjunction with this protozoan were sometimes normal and infective to mice but often were shrivelled and abnormal in many respects.

The hexacanth embryos upon entrance into the haemocoele of the insect, as stated before, remain upon the surface of the gut for a brief period while growth takes place. Soon, however, most of them migrate into the surrounding haemolymph. Dissected and sectioned material shows that the cysticercoids do not encyst in muscle or other host tissue but may become embedded among the abundant fat bodies; or, as more often seems to be the case, they are merely suspended in the haemolymph. Fully formed cysticercoids are usually present in the insect's abdomen, seldom are they encountered in the thoracic region. Pupation has no apparent effect upon development of the cysticercoid. Thus a larval insect can acquire the infection and thereafter remain infected through metamorphosis to the adult stage even though the adult has food habits which prohibit infection. An example of this is the Indian meal moth.

The number of cysticercoids harbored by a single insect may vary from one to at least two hundred and very possibly more. Heavily infected individuals were not encountered in the sectioned material, so counts from this material were smaller than those from living insects. One dissected *Trogoderma* larva was observed to contain 174 cysticercoids within the area of a cover slip three-quarters of an inch in diameter.

The percentage of *Trogoderma* larvae which became infected with cysticercoids varied from zero to almost 100 percent in experimental lots. Although the factors regulating infectivity of the insects were not studied it appears that the physiological state of the insect, its age, the condition of the infective meal, and the method of offering the tapeworm eggs are certainly involved. Viability of the eggs would also be an important factor but in all cases an attempt was made to use fresh material.

By feeding *Trogoderma versicolor,* from known infected lots, to mice it was possible to determine the number of days required for the cysticercoids to become infective, how long they remained viable within the insect, and what numbers of mice acquired the infection. Data for these studies were collected from a series of 36 experiments carried out with various purposes at room temperature throughout the year. Temperature is known to have a marked effect upon the life of the insect host, the larval stage being greatly prolonged by temperatures lower than optimum. There is some evidence that cysticercoids within the insect are also affected by temperature although this factor was not studied in a controlled manner. During cold months immature cysticercoids were found after 20 days within the insect while in warm weather, as has already been mentioned, cysticercoids appearing to be morphologically mature were found as early as nine days after infection of the insect. However, other factors regulating the rate of growth may be present since in many cases cysticercoids within a single insect were not equally developed. Cysticercoids have been shown to develop within the insect and become infectious to mice in a minimum of about 18 days. This was determined by exposing larval insects to tapeworm eggs, removing them to clean food two days later and feeding them to mice 18 days after the initial exposure. Mice proved negative at autopsy in similar experiments when fed insects held for 7, 10, 12, and 14 days after exposure to eggs. Tapeworms were recovered in 25 out of 29, or 86 per cent, of the remaining mice in this series which received insects exposed to eggs and held for various periods ranging from 18 to 143 days. The insects held for 143 days, as well as others in the series, had been infected in the larval stages and passed through metamorphosis to the adult stage, showing that metamorphosis has no effect upon the development and infectivity of the parasite.

The rôle of other species of insects as intermediate hosts has been discussed but the infectivity of the cysticercoids, if present, has not been studied so completely as in *Trogoderma.* Lots of the following species of insects, exposed to an infective meal, were fed to mice with the results as follows: nymphs of *Blatta orientalis* were fed to one mouse which remained uninfected; larvae of *Attagenus piceus* were fed to two mice, and both developed tapeworms; larvae of *Anthrenus verbasci* were fed to one mouse which remained uninfected; adults of *Tribolium confusum* were fed to seven mice, three of which developed tapeworms; larvae of *Tenebrio molitor* and *T. obscurus,* were fed to three mice, all of which remained uninfected; larvae of *Plodia interpunctella* were fed to one mouse which developed tapeworms. Two mice fed gravid proglottids and three mice kept continually with infected mice failed to become infected. It should be noted that all the above mentioned species of insects which were offered infective meals but failed to show cysticercoids on subsequent examination also failed to produce adult tapeworms when fed to mice. In those insects in which cysticercoids were found, all but *Anthrenus verbasci* successfully

transmitted the tapeworms. This exception has little significance as only two unexamined larvae were fed to one mouse due to the limited supply of these insects. There is no evidence to indicate that fully formed cysticercoids are not infective regardless of the intermediate host in which they occur.

The adult tapeworm.—The development of the tapeworm within mice was observed at various intervals from one hour until gravid proglottids were passed. A mouse was fed three infected *Trogoderma* larvae and an examination was made one hour later upon the stomach and first three inches of the small intestine. No evidence of the cysticercoides was found in the stomach, although setae and other chitinous parts of the insects were present. Four cysticercoids were observed in the duodenum. These were similar to cysticercoids dissected from insects in that the scolices had not yet evaginated; however, they were more active in their movements among the villi. In another mouse, examined 24 hours after the infective meal, a very young worm was observed which closely resembled the evaginated cysticercoid (Fig. 9). This tapeworm measured 2.9 mm long by 0.72 mm wide in an extended condition and tapered gradually from a rather broad scolex to a bluntly rounded posterior end. The suckers measured 0.54 mm across and the distinct cuticula was 36 μ in thickness. This worm, as in the case of all young stages, was capable of extreme flexibility, contracting to almost a spherical form and extending to a ribbon-like band. The scoleces continually protruded and contracted, grasping at the surfaces of the villi for attachment (Fig. 13). The large calcareous bodies of the cysticercoid were rather evenly distributed in the body of the worm and dorsal and ventral excretory ducts were well defined.

The growth of the worm continues, with an increase in length and general size, in complexity of the excretory vessels and in the appearance of segmentation. The large calcareous bodies of the cysticercoid and the embryonic hooks are retained in the end proglottids and are passed off before proglottids containing eggs are passed. A tapeworm four days within the mouse measured 7.1 mm long by 0.95 mm wide; after seven days worms ranged up to one centimeter; at 11 days they were two centimeters; at 13 days they were taken 2.5 cm long. By 21 days tapeworms 5.5 cm long were taken showing eggs forming in the younger segments but no eggs in the end proglottids. Tapeworms taken after 23 days within a mouse measured up to 7.5 cm long, which is about the average length of the mature worms. Gravid proglottids were passed by two other mice which were infected for 23 days and those from one of these mice proved infectious to *Trogoderma* larvae. Tapeworms gradually migrate further down the small intestine, mature worms occurring most frequently in the small intestine about five to seven inches below the stomach, but they may extend almost to the caecum.

Frequently mice infected for as long as a month showed enormous numbers of minute tapeworms measuring from less than one up to three centimeters long. These immature worms were usually present in clusters in the small intestine and often a fully mature specimen was found among them, or more usually, lower down in the intestine. Since these mice had been fed infected insects only once, these smaller tapeworms had lagged in their development.

In early experiments one rat fed *Trogoderma* larvae and four rats fed *Tribolium* adults, from infected lots of insects, failed to develop tapeworms. In later experiments, after the mouse-insect cycle was established, four rats were fed *Trogoderma*

larvae and in every case *Oöchoristica ratti* developed. Six adult tapeworms taken from rats ranged from 6 to 9 cm in length and 10 from mice ranged from 4.5 to 12 cm long. The average length of these worms from each group was approximately 8 centimeters.

SUMMARY

Mice inhabiting the Natural History building, Urbana, Illinois, U.S.A., were found to harbor *Oöchoristica ratti* Yamaguti and Miyata, 1937, a cestode of the order ANOPLOCEPHALA hitherto reported only from rats taken on ocean vessels in Japan.

Studies on the life cycle of this tapeworm revealed that both laboratory mice and rats may serve as the definitive host. The life cycle cannot be completed by direct infection of the definitive hosts but requires various species of insects to serve as intermediates. Insects which were found capable of serving as intermediate hosts are: *Trogoderma versicolor, Attagenus piceus, Anthrenus verbasci, Tribolium confusum, T. ferrugineum, Tenebroides mauritanicus,* and *Plodia interpunctella.* Insects tested but found unable to transmit the parasite are: *Blatta orientalis, Tenebrio molitor* and *T. obscurus.* Oribatid mites believed to be of the genus *Galumna* also failed to become infected.

Onchospheres of *Oöchoristica ratti* ingested by the insect are freed of their outer shell and penetrate the ventriculus within 48 hours, whence they gain entrance into the haemocoele. Here they become embedded among the fat bodies of the host or remain free in the haemolymph. Growth and development rapidly occur and cysticercoids are produced which become infective to mice or rats by at least the 18th day within the insect. Cysticercoids remain infectious at least as long as 143 days. They remain infectious throughout the insect's metamorphosis and life span. Development of the tapeworm within the mouse was studied at intervals from the time of ingestion of the cysticercoid until gravid proglottids containing infectious eggs were passed; a period of 23 days.

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Explanation of Plate

All figures concern *Oochoristica ratti* and are free hand drawings except Figs. 5, 10, 12, 15, and 16 which were drawn with the aid of a camera lucida. Figures representing forms developing in *Trogoderma versicolor* larvae concern a single experiment in which these insects were exposed to eggs of the tapeworm on July 16, 1940, and were removed to clean food four days later. This is the experiment from which unusually rapid development of the cysticercoid is described in the text.

Fig. 1. Egg.

Fig. 2. Embryonic hook.

Fig. 3. Hexacanth embryo four days within a *Tribolium* larvae showing elliptical areas described in text.

Fig. 4. Hexacanth embryo lying between circular muscle bands on the surface of the mid-gut of a *Trogoderma* larvae dissected on July 20, 1940, four days after initial exposure of the insect to infection.

Fig. 5. Embryo from a *Trogoderma* larva showing little cellular differentiation found on July 21, 1940, five days after initial exposure.

Fig. 6. Embryo showing beginning of cellular differentiation probably representing scolex formation found in a *Trogoderma* larva on July 23, 1940, seven days after initial exposure.

Fig. 7. Fully developed cysticercoid with the scolex shown somewhat diagrammatically found in a *Trogoderma* larva on July 25, 1940, nine days after initial exposure.

Fig. 8. Evaginated cysticercoid dissected from a *Trogoderma* larva on July 27, 1940, 11 days after initial exposure.

Fig. 9. Young tapeworm four days within a mouse.

Fig. 10–11. Fully developed cysticercoids surrounded by a membrane of unknown origin in *Trogoderma* larvae dissected on July 28, 1940, 12 days after initial exposure.

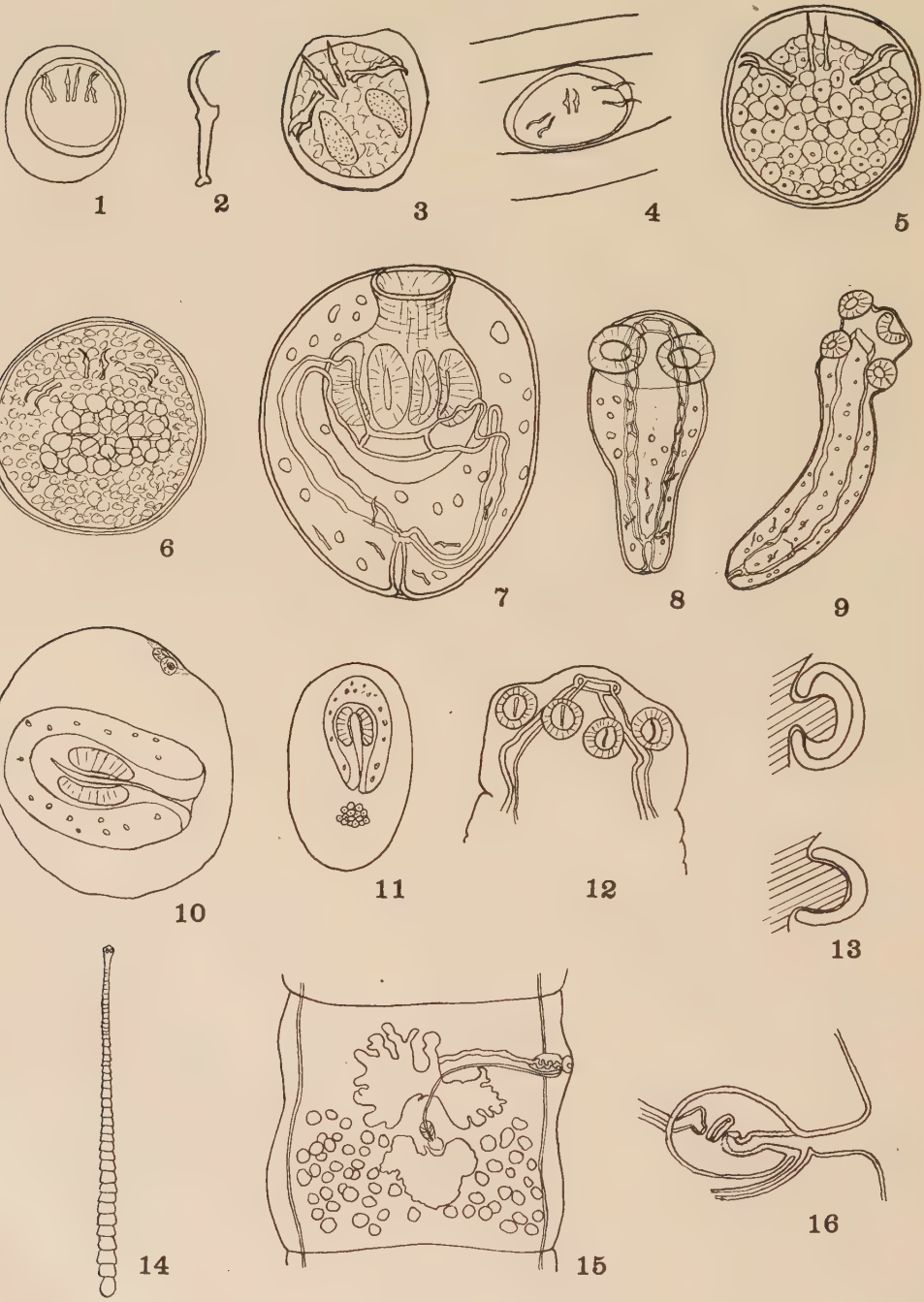
Fig. 12. Scolex of tapeworm 21 days within a rat showing the excretory ring.

Fig. 13. Outline drawings representing mechanism by which the suckers of the young tapeworm shown in Fig. 9 attached themselves to the surfaces of villi.

Fig. 14. Adult tapeworm.

Fig. 15. Mature proglottid.

Fig. 16. Genital atrium shown in transverse section.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RESEARCH NOTES

OCCURRENCE OF *CONIDOPHRYS PILISUCTOR* ON *COROPHIUM ACHERUSICUM* IN CALIFORNIAN WATERS

During the course of studies on the ectocommensal protozoans of the marine, wood-boring isopod, *Limnoria lignorum* (Rathke), a *Conidophyrs* (Protozoa, Ciliata) occurred from time to time attached to the bristles of the isopods. Subsequently the writers found *Corophium acherusicum* (Costa) inhabiting the galleries of the *Limnoria* in small numbers at Newport Bay, Orange County, California. Chatton and Lwoff (1934, Compt. Rend. Acad. Sc. **199:** 696–699) described *Conidophrys pilisuctor* from *C. acherusicum* at Sète and l'Etang de Thau, France. The Newport Bay *C. acherusicum* hosts are heavily infested with a protozoan which differs in no significant respect from *C. pilisuctor.* Individuals occur only on the structures Chatton and Lwoff call secretory hairs. All of Chatton and Lwoff's (1936, Bull. Biol. Fr. Belg. **70:** 86–144) stages (lacrymoid and spheroid trophonts and cylindrical stages) except the free-swimming infective individuals are represented in the writers' material. The *Conidophrys* on the *Limnoria* is also *C. pilisuctor,* but on this host the infestations are light.

As the amphipod host of this ciliate is cosmopolitan (Mr. Clarence R. Shoemaker, *in litt.;* Crawford, 1937, J. Mar. Biol. Assn., U. K. **21:** 589–630), *Conidophrys pilisuctor* may be presumed to have a wider distribution than the two localities from which it has been reported. The writers have observed other *Conidophrys,* undescribed, on marine amphipods from Plymouth, England, California, and Baja California. They found none of the ciliates on *Corophium acherusicum* or on *C. baconi* Shoemaker taken from fouling masses on a fixed channel marker in Los Angeles Outer Harbor.

As Kirby (Relationships between certain Protozoa and other animals, in Calkins and Summers Protozoa in Biological Research, New York: Columbia University Press, 1941) has pointed out, the familial name Pilisuctoridae proposed by Chatton and Lwoff and based on the specific name of their first-described species, is formed contrary to the International Rules of Zoölogical Nomenclature. Conidophryidae is the correct form of the name of the family.

The writers are indebted to Mr. Clarence R. Shoemaker, who identified the amphipods examined.—J. L. Mohr and J. A. LeVeque, *Department of Zoölogy, University of Southern California.*

THE OCCURRENCE OF *ASCARIS LUMBRICOIDES* L. 1758 IN THE MUSKRAT, *ONDATRA ZIBETHICA* L.

During November, 1947, carcasses of 21 muskrats collected near Champaign, Ill., were made available to the writers for parasitological examination. Helminths encountered were: *Hymenolepis* sp., one animal infected with four worms; *Taenia taeniaformis* (Batsch 1786), four animals infected, total of five cysts; *Echinostomum revolutum* (Frölich 1802), two animals infected, 11 specimens recovered; *Ascaris lumbricoides,* two animals infected, one of which harbored a male worm 150 mm long, and the other, a female 50 mm long. In addition, while one of us (J.D.T.) was serving as assistant, autopsies by students in Dr. L. J. Thomas' helminthology class revealed one of about 15 muskrats infected with a single immature *Ascaris* sp., probably *A. lumbricoides.* Thanks are expressed to Mr. B. R. Mestel and to Mr. Q. H. Pickering for allowing us to examine the first mentioned group of hosts, to Mr. R. J. Miller for collecting the second group, and to Mr. Bert Babero, finder of the third ascarid.

Reports of *A. lumbricoides* in rodents of the family Sciuridae have been discussed by Rausch and Tiner (in press, Am. Midl. Nat. 1948). Since the present report apparently constitutes a new record for the occurrence of *A. lumbricoides* in a member of the Cricetidae a careful study was made of the single mature male recovered in order to morphologically differentiate our specimen from *Ascaris columnaris* Leidy 1856. The male from the muskrat was found to have between 56 and 61 pairs of precloacal papillae, equal spicules 1.30 mm long, and an evenly striated cuticle, excepting papillae, both before and behind the cloaca. *Ascaris columnaris* males from a raccoon taken 2 miles north-east of Urbana-Champaign, Ill. had about 40 pairs of precloacal papillae and spicules 0.8–0.9 mm long. Roughened patches were present on the cuticle of *A. columnaris* directly anterior and posterior to the cloacal opening, as described by Goodey and Cameron (1923, J. Helminthol. **1:** 1–8).—Jack D. Tiner and Ta Hsiung Chin, *Vivarium Building, University of Illinois, Champaign, Ill.*

ABSENCE OF *TRICHINELLA SPIRALIS* IN RATS IN PUERTO RICO

Infections with *Trichinella spiralis* have not been reported from man or any other host in Puerto Rico. Bachman et al (1934, Am. J. Hyg. **20**: 415–423) reported negative findings on examination of the voluntary muscles and diaphragms of 300 rats collected in the city of San Juan, Puerto Rico. Collections of rats in connection with other studies have recently afforded further opportunity to investigate this problem. The diaphragms of 418 rats collected at random from San Juan and Santurce during the period of September 1946 to August 1947 were examined for larvae of *T. spiralis*. The whole diaphragm was examined in each case by the compression technique. No *Trichinella* larvae were observed in any of the tissues examined. The species of rats involved were as follows: *Rattus norvegicus*—179; *Rattus rattus rattus*—106; *Rattus rattus alexandrinus*—58; *Rattus rattus frugivorus*—75.—GEOFFREY JEFFERY, *Communicable Disease Center, U. S. Public Health Service, and* JOSE OLIVER-GONZALEZ, *Department of Medical Zoölogy, School of Tropical Medicine, San Juan, Puerto Rico.*

AMERICAN SOCIETY OF PARASITOLOGISTS

Thirty-Seventh Council Meeting, Chicago, Illinois

December 29, 1947

The meeting of the Council of the American Society of Parasitologists was called to order by President Harley J. Van Cleave at 7:30 PM, December 29, 1947, in the Congress Hotel, Chicago, Illinois. Past-Presidents W. W. Cort and D. H. Wenrich and the following members of Council were present: T. W. M. Cameron, G. R. Coatney, J. T. Culbertson, C. G. Huff, G. F. Otto, C. B. Philip, R. M. Stabler, H. W. Stunkard, H. J. Van Cleave, W. H. Wright, and M. D. Young. In addition, W. M. Reid was also present by invitation for the presentation of a special report.

The regular order of business was set aside in order that Dr. Reid might present his report as the Society's representative to the Committee on Visual Instruction in Microbiology of the Society of American Bacteriologists. Dr. Reid's report recommended that the Society create a Committee to encourage the editing and production of parasitological motion picture films to work in collaboration with Dr. H. E. Morton of the Society of American Bacteriologists, and that, after a year or so, this committee also take over the function of abstracting films. It was moved by Dr. Otto that such a committee be created, appointment to be made by the retiring President of the Society. The motion carried.

The regular order of business was then taken up.

I. Reports of Officers

1. *Secretary (J. T. Culbertson):* As of December 24, 1947, there were 621 members of the Society, of whom 559 lived within and 62 lived outside continental United States. Of these, 30 persons were delinquent for dues for 1947, leaving a net membership in good standing of 531 domestic and 60 foreign persons for a total of 591 active members. It is a good sign for the future that the present active membership of the Society is the highest on record. Sixty-eight persons have been elected to membership during the calendar year 1947 to December 24, of whom 65 lived within and 3 lived outside continental United States. The names of six additional persons will be presented to Council for election to the Society later in this meeting. The number of electees during 1947 is the highest for any year, excepting 1945, since 1929.

Since organization of the Society, 1416 persons have been elected to membership. The present active paid up membership (591) represents 41.7% of the total number of electees during the 24 years of the Society's existence.

During the year, the following members died: Rudolf Glaser, Seymour Hadwen, Charles A. Kofoid, Samuel Morris, and Harry Plotz.

The report was accepted and placed on file.

2. *Treasurer (R. M. Stabler):* The Treasurer's report for the fiscal year 1947 (December 1, 1946, to December 1, 1947) follows:

Receipts

Balance on hand Dec. 1, 1946		$ 5,006.91
Collected during the current year:		
493 members dues applying 1947	$1,969.05	
18 members dues applying 1948	66.00	
5 members dues applying after 1948	39.00	
6 dues in arrears	88.00	
65 applicants	261.00	
		$2,423.05
520 subscriptions to Vol. 33	2,452.25	
104 subscriptions to Vol. 34 and after	527.25	
Sales of individual numbers (Vol. 33)	18.75	
		2,998.25

Back volumes and numbers	572.15		
Advertisements	174.61		
Author's charges	85.19		
Quindex	37.65		
Interest on savings account	8.70		
Gift. Amer. Found. for Trop. Med.	1,000.00		
Miscellaneous	58.70		
		1,937.00	
			7,358.30
			$12,365.21

Debits

Printing and handling journal			
Volume 32	$1,950.36		
Volume 33	3,612.95		
		$5,563.31	
Expenses in office of Science Press	45.86		
Expenses in office of Chairman of Editorial Committee	329.45		
Expenses in office of Secretary	231.06		
Expenses in office of Treasurer	648.07		
Expenses in office of Custodian of Back Issues	108.80		
		1,363.24	
			$ 6,926.25
Balance on hand December 1, 1947			$ 5,438.66
			$12,365.21

The report was accepted subject to audit, and placed on file.
Audited and Found Correct, December 29, 1947,
by Auditing Committee:
(signed) W. H. WRIGHT
(signed) T. W. M. CAMERON.

II. REPORTS OF CUSTODIANS AND COMMITTEES

1. *Custodian of the Princeton Secretarial Fund (N. R. Stoll):* The report, covering the period December 1, 1946, to December 1, 1947, follows:

Savings bank balance, December 1, 1946	$1,058.14
Interest credited	10.60
Savings bank balance, December 1, 1947	$1,068.74

The report was accepted, subject to audit, and placed on file.
Audited and found correct, December 29, 1947,
by Auditing Committee:
(signed) W. H. WRIGHT
(signed) T. W. M. CAMERON.

2. *Chairman of the Editorial Committee (H. W. Stunkard):* The issues of the Journal of Parasitology, Volume 33, 1947, constitute the essential part of the report of the Editorial Committee.

The editorial policy has continued without change. The sum of $100 is allowed as the amount permitted an author, without overcharge, for publication. Costs exceeding that amount are shared equally by the author and the Society. This arrangement permits a paper of approximately ten pages, with one page used as tabular material, or illustration by either ink etching or halftone reproduction. Inserts on specially coated paper for halftone reproduction are charged to the author. In accordance with the action of Council authorizing the committee to accept manuscripts of any length, providing they are otherwise desirable, longer papers have been accepted and final charges have been adjusted with authors or the institutions they represent. As a result of careful editing, costs of authors' alterations have been kept to a minimum and there have been no author charges for excessive alterations during the past year.

Last year all printing charges were increased 10% and beginning January 1st, 1948, a further increase of 20% will be made. If the Journal is to continue its present schedule of printing approximately 600 pages per volume, it is imperative that subscription charges be increased. To meet the existing situation, the receipt of $1000 was announced from the American Foundation

for Tropical Medicine, Inc., which sum was transmitted to the Treasurer. The money was granted without restriction, to enable the editorial committee to accept and publish papers that otherwise would necessarily be rejected or delayed. For the past year advertising costs were increased by about 20% and the present schedule is as follows:

	One insertion	One yr. 6 issues
Full page	$18.00	$80.00
Half page	10.00	45.00
Quarter page	7.50	30.00

Exchange advertisements are used to fill an issue when space permits, and are maintained with Biological Abstracts, Transactions of the American Microscopical Society, the American Journal of Tropical Medicine, the Journal of the National Malaria Society, and the American Veterinary Medical Association. These publications, in turn, advertise the Journal of Parasitology, and such advertising brings the Journal to the attention of a wide list of readers. The five numbers issued so far this year, consist of 447 pages and the December Supplement contains 48 pages, a total of 495 pages published in 1947. Corrected galley for the December issue was sent to the Press on December 19th. The five numbers, February to October, inclusive, contain 66 articles by 85 authors and 16 research notes by 23 authors.

The cost of the five issues of the Journal of Parasitology, Volume 33, which had already been paid for, is:

February issue (1400 copies)	$885.11
April issue (1600 copies)	960.31
June issue (1600 copies)	908.10
August issue (1600 copies)	859.43
October issue (1600 copies)	699.28

Dr. Coatney moved that the report be accepted, with thanks to the Chairman of the Editorial Committee. Motion carried.

3. *Custodian of Back Issues (G. F. Otto):* The need for reprinting certain issues or volumes of the Journal which are now low in stock was emphasized. It was pointed out, for example, that of the 15 volumes published since the Society acquired the Journal (Volumes 18 to 33), only single sets of 3 volumes remain. No issues were reprinted during the past year but, if complete sets are to be maintained for sale, at least 4 and probably 10 issues will have to be duplicated in 1948. It was recommended that funds be sought to reproduce at the earliest possible date all numbers from Volume 19 to date which are now represented by 10 or fewer copies in stock. At the rate of $0.013 per page for the 10 issues previously referred to, the total cost would be at least $2262.00 (874 pages) for 200 copies each. The report was accepted as read.

During discussion it was suggested that the American Foundation for Tropical Medicine, Inc., be approached for support in reprinting the back issues of the Journal now low in stock. It was also suggested that since the Society had a considerable cash reserve at the moment and since the sale of back issues could be expected to pay for their reprinting, that the Society's present funds be used to reprint at least some of the issues now low in stock. Implementing this second suggestion, Dr. Stabler moved that the Treasurer be empowered to transfer to the Custodian of Back Issues a fund of $1500 for the reprinting of back issues, and that hereafter receipts from sale of back issues be added to this fund. The motion carried.

Dr. Stabler urged that, to alleviate the excess work in the Treasurer's office, the Custodian of Back Issues be permitted to handle the sale of back issues independently of the Treasurer. Dr. Otto pointed out that if it were desired that the Custodian become a separate officer of the Society, the Constitution of the Society would have to be amended. Dr. Coatney moved that the Constitution be amended to create a new officer of the Society—namely, Custodian of Back Issues of the Journal of Parasitology—who would be empowered to act independently of the Treasurer. The motion carried. The motion did not specify whether this officer was to be elected or appointed, nor did it indicate the term of office, whether or not this officer was to be a member of the Society Council, or whether this officer was to be bonded. The Secretary was directed to present the amendment as voted by the Council to the Society membership at its general business meeting on December 30, 1947.

4. *Auditing Committee (W. H. Wright and T. W. M. Cameron):* The reports of the Treasurer and of the Custodian of the Princeton Secretarial Fund were certified to be correct. The report of the Auditing Committee was accepted.

5. *Special Standing Committees:* The Committee on Nomenclature (E. W. Price, Chairman) and the Committee on Strains of Avian Malaria (C. G. Huff, Chairman) reported no activity during the year 1947.

III. Reports of Representatives

1. *To Council of A.A.A.S. (W. H. Wright and G. R. Coatney):* It was reported that the A.A.A.S. would hold its next meeting in Washington, D. C., during September, 1948, celebrating the centennial of the founding of the American Association for the Advancement of Science. Programs of submitted papers were, probably, not to be invited from the various affiliated societies, contrary to past years, although such societies would be assisted in arranging such programs in the week before or the week following the centennial celebration. The opinion was expressed that it might not be possible in future meetings to provide for programs of affiliated societies.

2. *To Council of U.A.B.S. (D. L. Augustine and R. M. Stabler):* No report was submitted.

3. *To Committee on International Congresses of Tropical Medicine and Malaria. (W. W. Cort):* The Congresses on Tropical Medicine and Malaria will be held May 10 to 18, 1948, in Washington, D. C. A general prospectus of the meeting was soon to be mailed to all members of the Society with invitation that they become members or associate members of the Congresses. Altogether 12 sections of the Congresses were being organized. Sections of particular interest to members of this Society were those on Malaria, Helminthic Diseases, Protozoal Diseases, and Medical and Veterinary Entomology. This Society is to be represented officially at the Congresses by Dr. C. W. Rees. The report was accepted.

4. *To National Research Council (E. W. Price):* The National Research Council, Division of Biology and Agriculture, was sponsoring the formation of the American Institute of Biological Sciences. The main purpose of the Institute was to represent the biological sciences in national and international matters. As need arose and as the governing Board directed, many specific services could be undertaken. The budget needed for the first year would amount to $26,500. To cover this, an assessment on various member societies of approximately one dollar per dues-paying member was anticipated, with allowance for persons who belonged to several participating organizations. The Council of the National Academy of Sciences on April 27, 1947, authorized the National Research Council to accept and administer funds for the formation of the Institute. It was stated that seven societies had indicated that they favored joining the Institute as charter members either with full membership or with reservations. An invitation that this Society become a charter member of the Institute was implicit. The report was accepted and placed on file.

Dr. Stunkard moved that the proposal that this Society accept charter membership be presented to the Society without recommendation, for discussion, with a vote of members to be taken on the issue at a later date by mail or, if advisable, at the next general business meeting. Motion carried.

IV. New Business

1. *Election of New Members:* The following persons were elected by Council to active membership in the Society: Donald East Hunt, Department of Parasitology, University of North Carolina, Chapel Hill, North Carolina; Reginald Frederick Lawrence, Director of the Natal Museum, Pietermaritzburg, South Africa; Charles Francis Smith, 1301 West Campus, Lawrence, Kansas; I. Barry Tarshis, Department of Entomology, University of California, Berkeley, California; Mary Jane Wegert, Department of Biology, William Jewell College, Liberty, Missouri; and James E. Williams, Department of Zoology, University of Michigan, Ann Arbor, Michigan.

2. *Place of Meeting in 1948:* Letters were read from the secretaries of the National Malaria Society, the American Society of Tropical Medicine, and the American Academy of Tropical Medicine inviting this Society to meet conjointly with them in 1948, probably in New Orleans early in December. The invitation was given extended discussion by Council. Correspondence was also considered opposing this Society meeting separately from A.A.A.S. Dr. Otto finally moved that since no meeting place for the Society was to be provided by A.A.A.S. in 1948, Council should recommend to the membership that the invitation to meet in 1948 with the three Societies named above be accepted, the precise time and place of the meeting to be decided by representatives of the four groups. The motion was seconded by Dr. Huff. The motion carried.

3. *Donations:* Dr. Stunkard moved that the Society donate ten dollars to the American Society of Naturalists to assist with the Biologists' Smoker. Motion carried.

Dr. Wright moved that the appeal from the Zoological Society of London for financial assistance be tabled. The motion carried.

4. *Modification of Scientific Program:* Correspondence with Dr. Brackett was read in which he appealed for modification of procedures for erecting the scientific program to permit greater apportunity for discussion. The Council failed to take action on the appeal because of uncertainties as to the type of meeting in 1948. A committee to inquire into the problem may be appointed later in the year.

5. *Nominations, Elections, and Appointments to Society Offices, etc.:*
   a. The following persons were nominated by Council for the designated offices in the Society: President for 1948, E. C. Faust; Vice-President for 1948, C. B. Philip; Secretary for 1948 and 1949, H. W. Brown; Councillors-at-Large for four years, C. G. Huff and P. D. Harwood.
   b. The following persons were elected by Council to the Editorial Committee for the five years, 1949–53: H. W. Stunkard (Chairman), W. A. Riley, and J. M. Andrews. W. W. Cort was elected Chairman of the Editorial Committee for 1948 to act for H. W. Stunkard, who is to be on sabbatical leave from his institution. The following persons were elected by the Council to the Editorial Board of the Journal of Parasitology: E. R. Becker, N. R. Stoll, and G. W. Wharton.
   c. President Van Cleave appointed, for a two-year term, the following persons as representatives to the Council of the A.A.A.S.: A. O. Foster and K. C. Kates.
   d. President Van Cleave appointed to the newly created Committee on Visual Instruction: M. S. Ferguson (Chairman) and W. M. Reid.
   e. President Van Cleave named to the Committee on Nomenclature: E. W. Price (Chairman), G. W. Wharton, and F. J. Denton.
   f. President Van Cleave recommended (in a letter to the Secretary dated December 31, 1947) that the Committee on Avian Malaria and on Common Names be discontinued. The recommendation will be referred back to the Council for action.

Respectfully submitted,
JAMES T. CULBERTSON, *Secretary.*

TWENTY-SECOND ANNUAL GENERAL BUSINESS MEETING

DECEMBER 30, 1947

The general business meeting of the Society was called to order by President H. J. Van Cleave at 2:00 PM, following the annual luncheon at the Stevens Building Restaurant, Chicago, Illinois. One hundred and sixty-six persons were present. The reports of the Secretary, Treasurer, Custodian of the Princeton Secretarial Fund, and Chairman of the Editorial Committee were read without comment. The report of the Custodian of Back Issues was given and the proposed amendment to the Society Constitution (to be voted on at the next general business meeting) was read. The proposed amendment, as approved by Council, would change the first paragraph of the article of the Constitution entitled "Officers" to read: "The officers of the society shall be a President and a Vice-President, who shall be elected for one year, a Secretary and a Treasurer, who shall be elected for 2 years; a Custodian of Back Issues of the Journal of Parasitology, who shall be elected for 3 years and members at large of the Council."

The report of the representative of the Society to the Council A.A.A.S. was read by W. H. Wright, that of the representative to the Committee on the International Congresses of Tropical Medicine and Malaria was given by W. W Cort, and that of the representative to the Committee on Visual Instruction in Microbiology of the Society of American Bacteriologists was presented by W. M. Reid.

Extended discussion followed the reading of the report of the representative to the National Research Council and the presentation to the Society of Council's action (see minutes of Thirty-Seventh Council Meeting) on the proposal the Society accept charter membership in the American Institute of Biological Sciences. Dr. Wenrich moved that the Society accept charter membership at the present business meeting. Arguments favoring Dr. Wenrich's move were offered by President Van Cleave, by Past-Presidents W. W. Cort and J. E. Ackert, and many others. Dr. Otto reminded members that affirmative action on the proposal would probably result in an increase in members' dues to the Society and Dr. Philip pointed out that the Society of American Bacteriologists had joined "with reservations"—i.e., by limiting the total dues assessed. The Motion carried on rising vote with only six dissenters, although some persons present did not vote. Accordingly, the Society becomes a charter member of the American Institute of Biological Sciences.

The election by Council of six applicants for active membership was announced by the secretary.

An amendment to the Constitution, recommended by the Council and previously presented to the membership by publication in the October issue of the Journal, was approved by the Society, without discussion. Accordingly, the article of the Constitution entitled "Dues" is to be amended to read: "The dues, to include subscription to the Journal of Parasitology, shall be five dollars the year for active members, unless changed by vote of the Society."

The proposal of Council that since no meeting place in 1948 was provided for the Society by A.A.A.S., that the Society accept the invitation of the National Malaria Society, the Ameri-

can Society of Tropical Medicine, and the American Academy of Tropical Medicine, was read to members. In the extended discussion which followed, it was brought out that the proposed arrangement did not involve severing the Society's affiliation with A.A.A.S. and that the proposal for a conjoint meeting with the inviting groups was for the year 1948 only. It was finally moved that Council action in accepting the invitation to meet in 1948 with the National Malaria Society, the American Society of Tropical Medicine, and the American Academy of Tropical Medicine, be approved. The motion carried.

The Secretary read the names of the persons nominated by Council to the Society offices. There being no nominations from the floor, it was moved and seconded that the Secretary cast one ballot for the slate of nominees. The motion carried. Accordingly, the officers for 1948 are: President, E. C. Faust; Vice-President, C. B. Philip; Secretary (to serve through 1949), H. W. Brown, and Councillors-at-large (to serve through 1951), P. D. Harwood and C. G. Huff.

The Secretary then read the names of the persons elected by Council to the Editorial Committee and to the Editorial Board of the Journal.

The Society voted to adjourn at 3:15 PM.

Respectfully submitted,

JAMES T. CULBERTSON, *Secretary.*

Announcement of Conference on Coccidiosis.

The New York Academy of Science is sponsoring a research conference on Coccidiosis to be held in the American Museum of Natural History, New York City on March 3 and 4, 1949. Investigators throughout the country who are interested in this subject are invited to attend. The program is being planned to cover the entire range of interests in coccidiosis. There will be an opportunity for the presentation of volunteer papers. The deadline of November 15 has been set for the submitting of the titles of papers to be presented at this conference. Further information can be obtained from Dr. Sterling Brackett, Organizing Chairman, American Cyanamid Company, 1937 West Main Street, Stamford, Connecticut.